bio-techne®

SIMPLE WESTERN
基本原理及实验操作

王娴婷 FAS
Tel: （+86）185-1660-6926
E-mail: xianting.wang@bio-techne.com

PROTEIN SIMPLE -- 创新蛋白质分析技术专家

Jess/Abby/Wes

全自动 Western

FluorChem

多功能成像

Ella

微流控全自动 ELISA

Milo

单细胞 Western

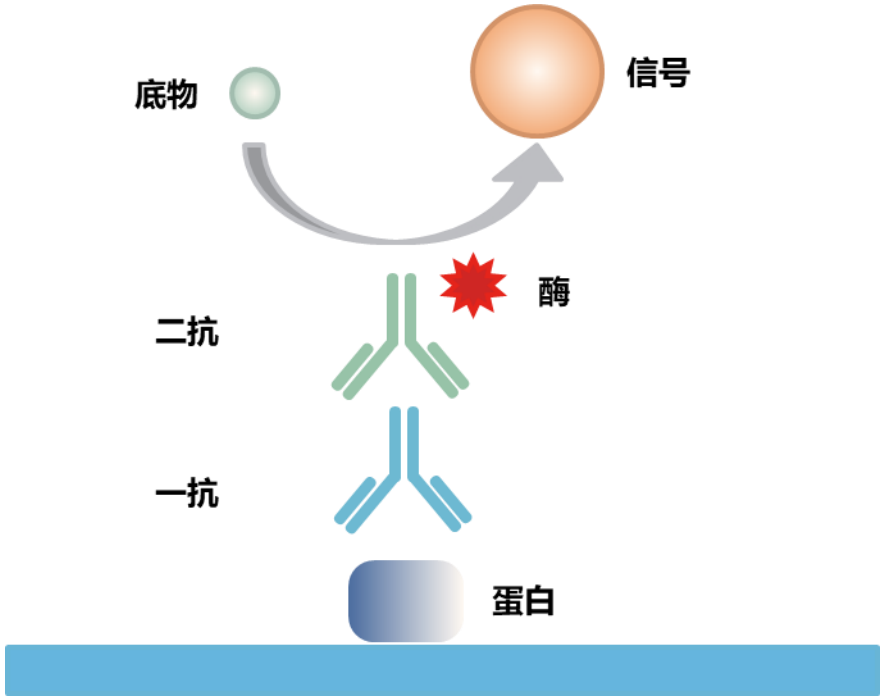
MFI

微流成像颗粒计数分类仪

Maurice

CE-SDS + icIEF 双功能 CE

免疫测定



典型的一抗结合目标蛋白的免疫测定。二抗结合一抗。二抗耦连酶，该酶能催化底物反应，产生能够检测的光信号。

- 任何使用抗体对混合蛋白中的靶分子或者目标蛋白进行检测的试验都叫**免疫测定**
  - 一些传统的免疫测定实验如：ELISAs 、Western Blots.
- 一抗：检测目标蛋白
- 二抗：与一抗结合，辅助检测
  - 一般耦连酶或者显色基团
- 抗体复合物和目标蛋白可以多种方式检测
  - 化学发光（ Chemiluminescence ）
  - 荧光 （ Fluorescence ）

当你在做 WESTERN BLOT 时

*图片来源于网络

当你搜索 WESTERN BLOT 时

【转】五年western blot 实验经验总结

https://www.douban.com/note/13641

2011-2-24 · 4 Western Blot实验关键 Weste

言，扩

的选择

【求助】Western blot内参actin结果重复不出来 - 经验共享 ...

https://bbs.antpedia.com/thread-228440-1-1.html

2013-6-1 · 标题:【求助】Western blot内参actin结果重复 不出来 暗香涌[使用道具] 三级 UID 108870 精华

离线 1 发表于

为什么同一批次提取蛋

https:

2013-8

血泪教训，Western blot史上最全避雷手册。 - * 乎

https://zhuanlan.zhihu

2020-9-3 · 提起Western

验，简称"玄学"。 话不多说

顺利利。 倾 尽 全 力...

你的Western Blo

https://www.jianshu.c

2020-8-24 · 做Western B

昨天的差异这

Western Blot详解 - 常见的问题指南（一） - 专区 - 生 * 谷

https://news.bioon.com/article/6005931.html

W. Western Blot 中抗体的重复应用问题 解答：抗体工作溶液一般不主张储存反复使用，但是如抗体比较珍

[实验经验]组织样本的western blot，这十点需要注意 - * 乎

https://zhuanlan.z

Western Blot详解 - 常见的问题指南（二） - 专区 - 生 * 谷

https://www.bioon.com/article/6005934.html

就重复性而言：尼龙膜可反复用于分子杂交，杂交后，探针分子可经碱变性被洗脱下来；硝酸纤维素膜不能重复使用；PVDF 膜可以重复使用。 Y. 在做Western Blot时，PBDF膜用甲醇浸泡的目的? 解答：PVDF

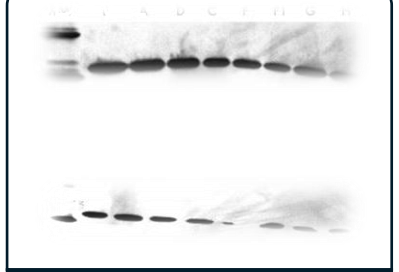
如何解决western blot半

https://wenda.guidechem.com/

2019-3-12 · 如何解决western blot半

显色的会影响条带的趋势，已把ECL均匀的滴在整张膜上，看见荧光后，曝光，得到一种趋势结果，然后，这张膜荧光渐渐黯淡，为了增加强度，重新再滴加ECL， 也覆盖的很均匀 ...

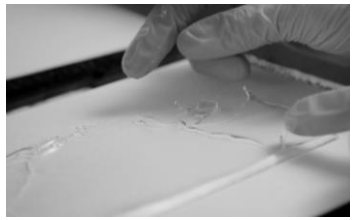
传统WB面临的挑战



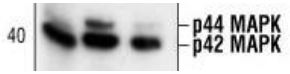
重复性差 1

- 5ug-20ug 总蛋白上样量

需要大量样本 2



大，小分子量蛋白 3



定量差 4

- 12-24 小时，手动操作多

周期长 5

传统WB面临的挑战

一直重复做WB

*图片来源于网络

MEET SIMPLE WESTERN

JESS ABBY WES

GEL-RUNNING AUTO
TRANSFER-FREE
BLOT-FREE
HANDS-FREE

超微量样品+自动化+定量

1. Simple Western 工作原理
2. Simple Western 优势及应用
3. Simple Western 实验操作简介

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SIMPLE WESTERN 工作原理

JESS ABBY WES

SIMPLE WESTERN 工作原理

化学发光检测

基于 CE-SDS 的免疫学分析

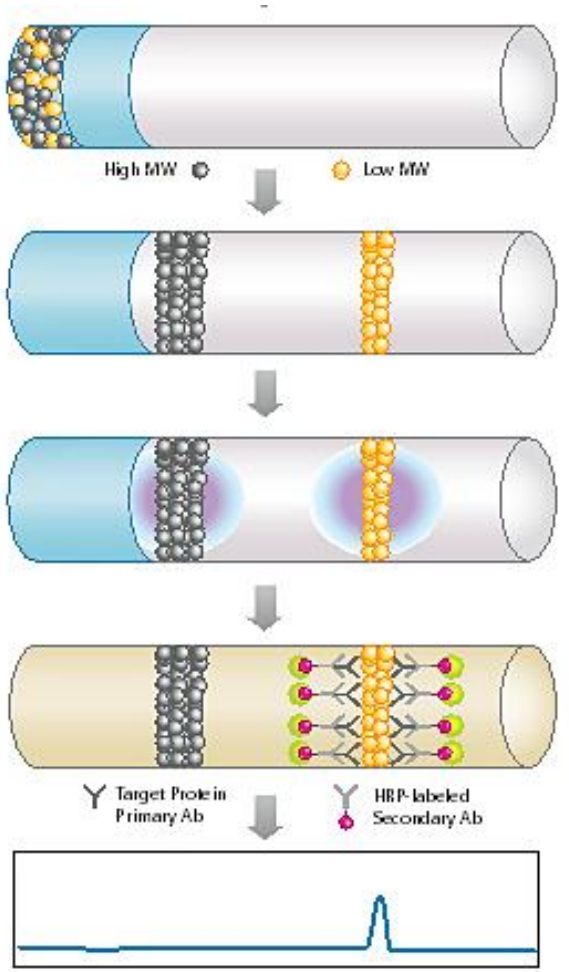
蛋白上样

蛋白分离

蛋白固定

免疫杂交

数据采集



所有在毛细管内的步骤都自动完成!

与传统 Western *不同*:

无需制胶（液态胶）

无需转膜（紫外激活交联）

无需人工孵育/清洗/压片

无需人工数据分析

MEET SIMPLE WESTERN

仪器型号	Wes	Abby	Jess
化学发光检测	√	√	√
荧光检测（IR/NIR）	-	-	√
总蛋白检测	√	√	√
总蛋白归一化	-	-	√
Replex	-	√	√
化学发光成像	-	-	√

工作原理——荧光/总蛋白归一化

制胶

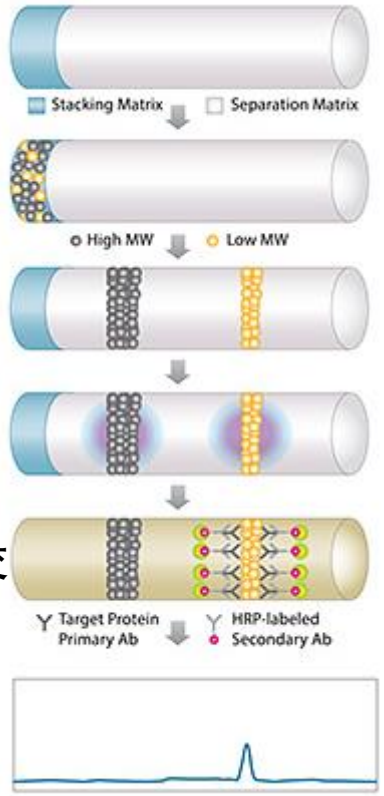
上样

分离

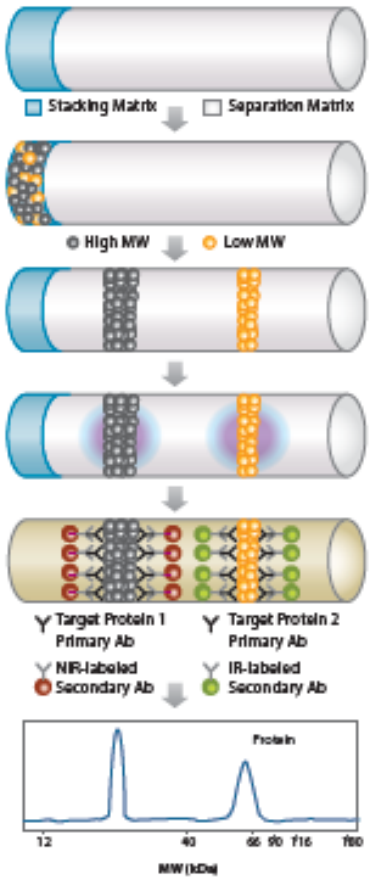
固定

免疫杂交

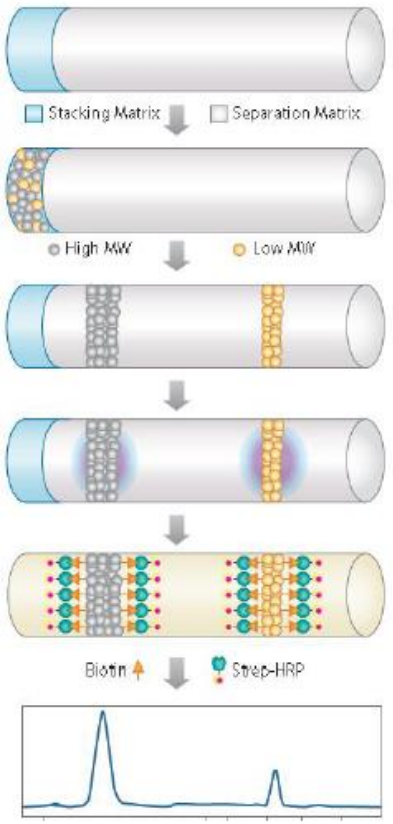
定量



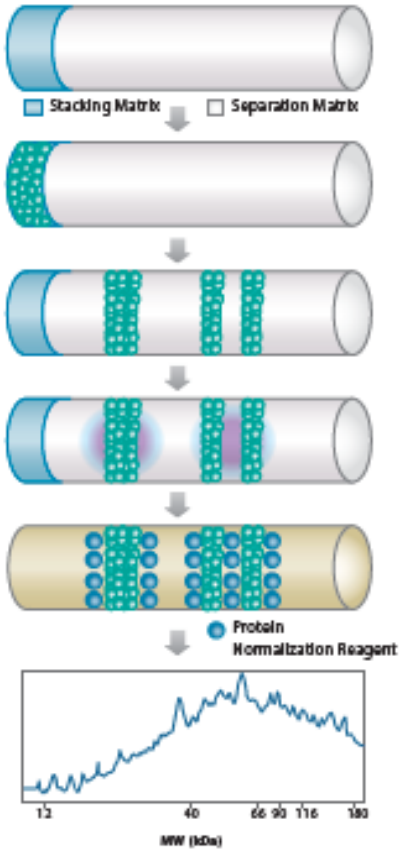
化学发光检测



荧光

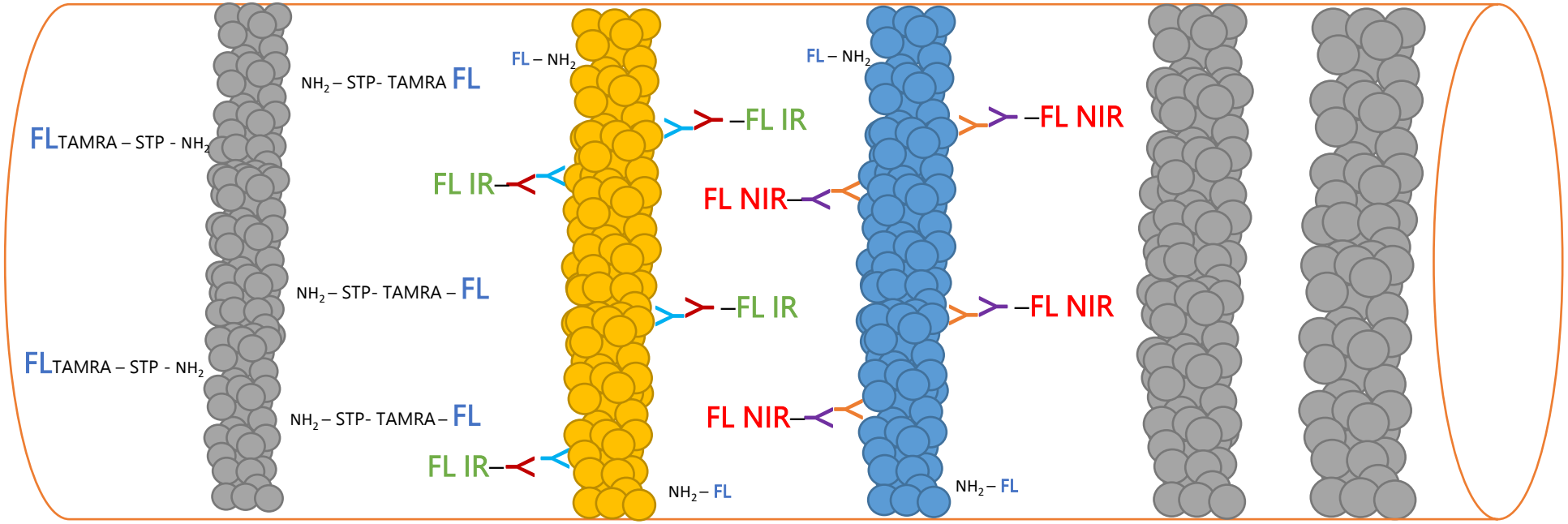


总蛋白检测



总蛋白归一化

总蛋白归一化检测



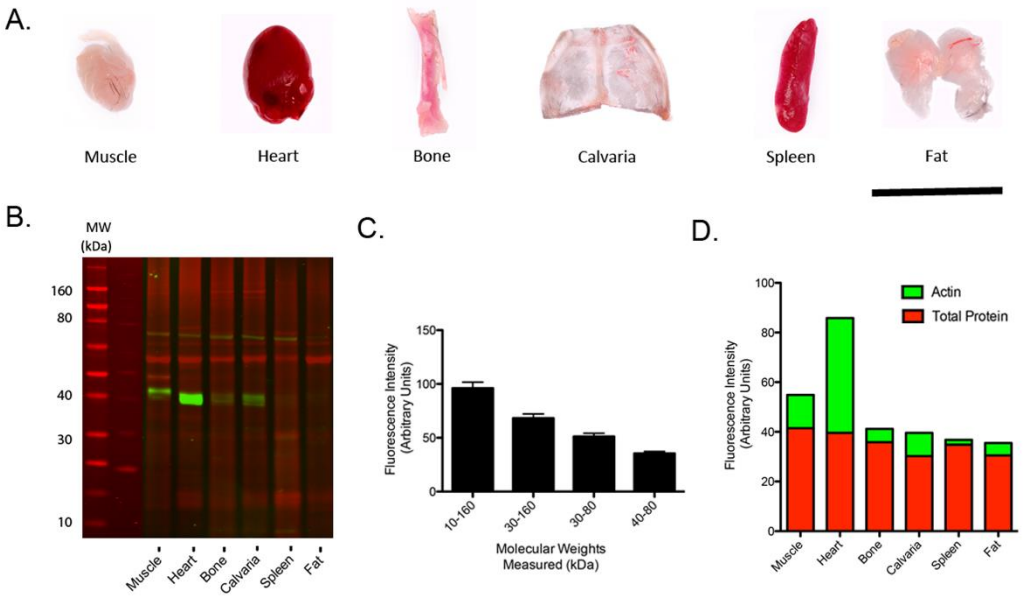
- 在 同一根 毛细管内
  - 基于荧光检测 （与IR和NIR的荧光染料不同）
  - 可与所有蛋白质结合的专利试剂
  - 只需加入一种试剂
- 可检测毛细管结合的总蛋白量
- 校正**批次内**不同毛细管之间蛋白上样量的差异
- 必须和免疫检测同时使用

PROTEIN MOLECULAR WEIGHT RANGE		
LYSATE CONCENTRATION	12–230 kDa	
	Stock Solution	Reconstitution Agent
0.2–1.2 mg/mL	50 µL	250 µL

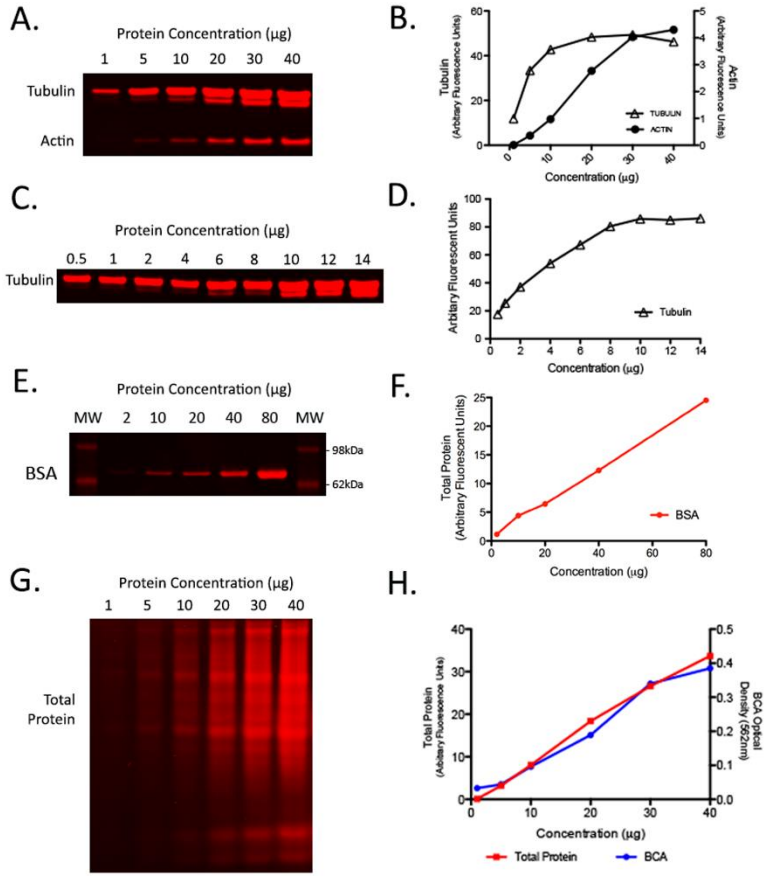
注意： -只能分析12-230kd的蛋白
-上样浓度0.2-1.2mg/ml
-不能单独检测

总蛋白归一化-WESTERN BLOT相对定量的趋势

内参蛋白受组织来源和实验处理的影响



内参蛋白的线性范围窄，无法满足所有靶蛋白的线性范围



Total Protein Analysis as a Reliable Loading Control for Quantitative Fluorescent Western Blotting,2013. doi:10.1371/journal.pone.0072457.

总蛋白归一化的应用实例

PNAS Proceedings of the National Academy of Sciences of the United States of America

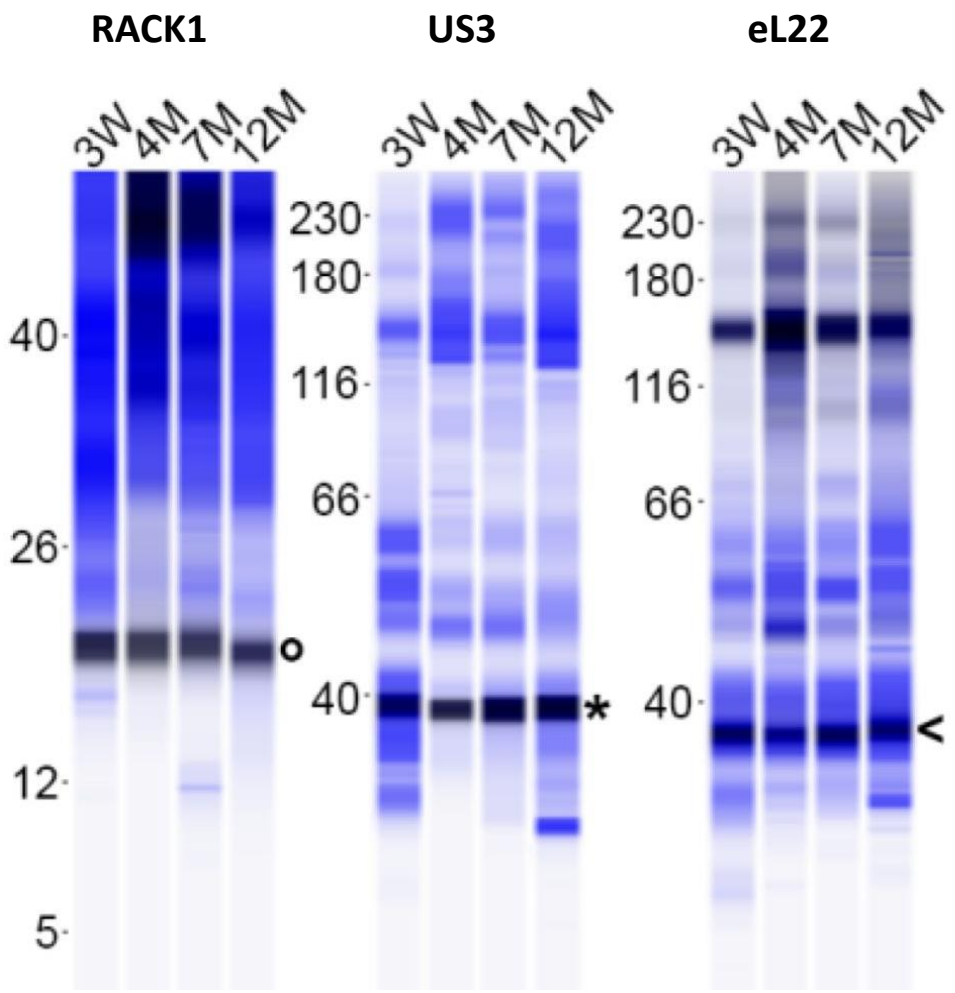
RESEARCH ARTICLE

Invariable stoichiometry of ribosomal proteins in mouse brain tissues with aging

Susan Amirbeigiarab, Parnian Kiani, Ana Velazquez Sanchez, Christoph Krisp, Andriy Kazantsev, Lars Fester, Hartmut Schlüter, and Zoya Ignatova

PNAS November 5, 2019 116 (45) 22567-22572; first published October 21, 2019
https://doi.org/10.1073/pnas.1912060116

利用Jess的“总蛋白归一化”作为loading control，减少了不同样品间内参不平带来的相对定量误差。



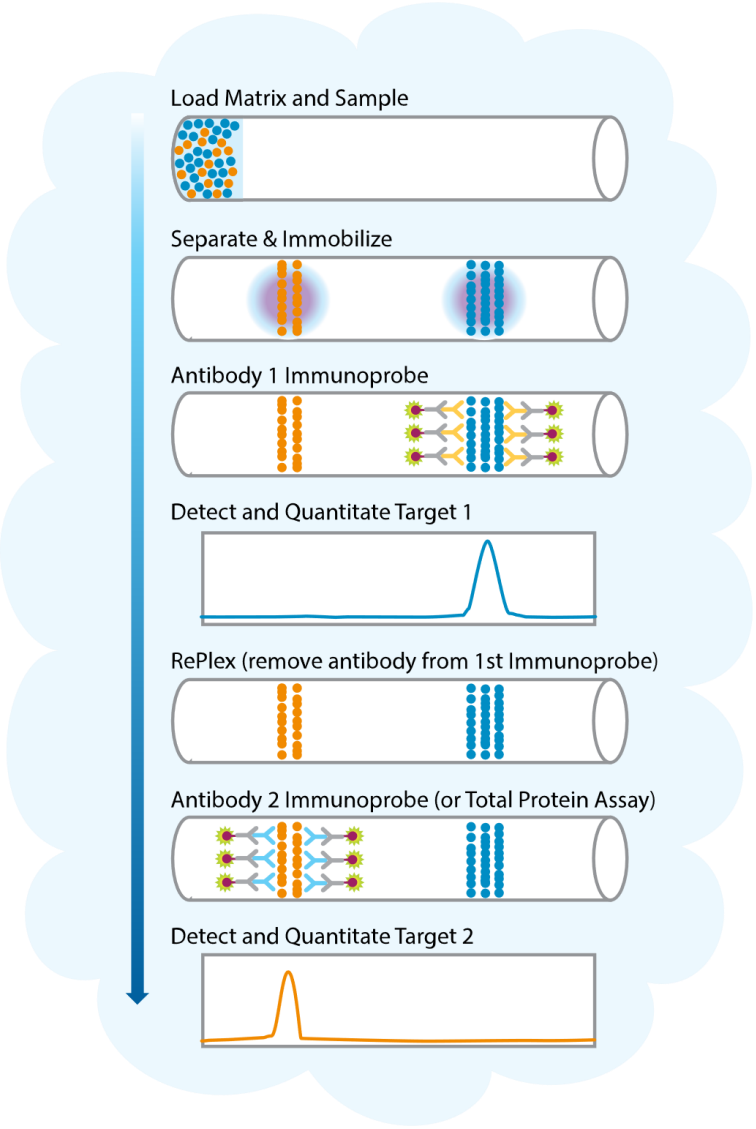
RePlex 定义

一根毛细管内进行两轮免疫学检测 (2 次连续检测，每轮只测1个靶点)

可用于化学发光、荧光及总蛋白检测

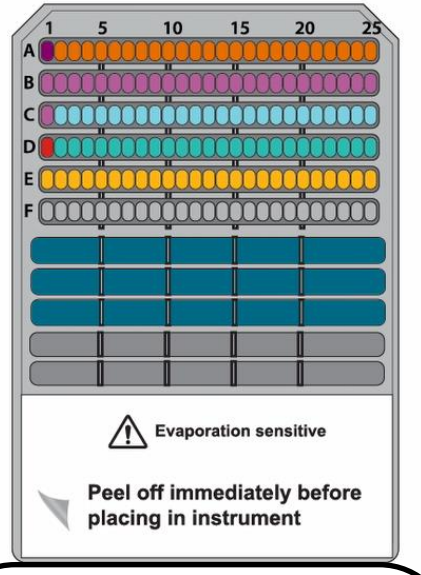
节省单个数据点检测费用

利用Simple Western技术独有的蛋白质与毛细管共价交联固定



您需要做的工作

1. 配制样品和试剂
- 加样
- 混匀
- 加热



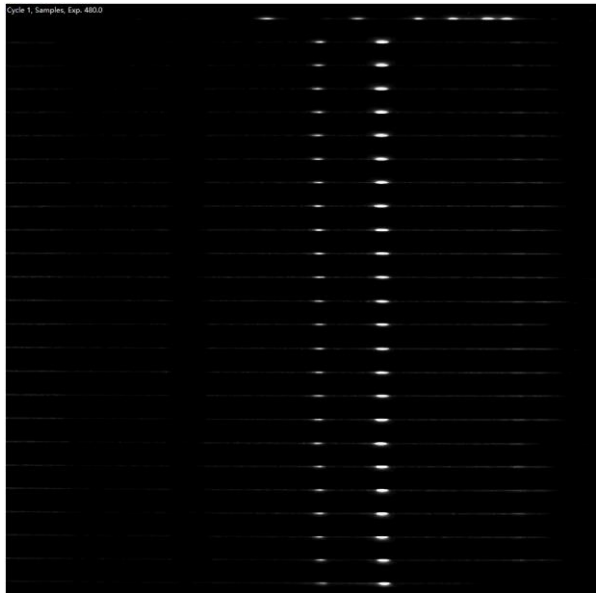
2. 加样
- 加marker
- 加样品
- 加封闭液
- 加一抗
- 加二抗
- 加发光液
- 加洗液

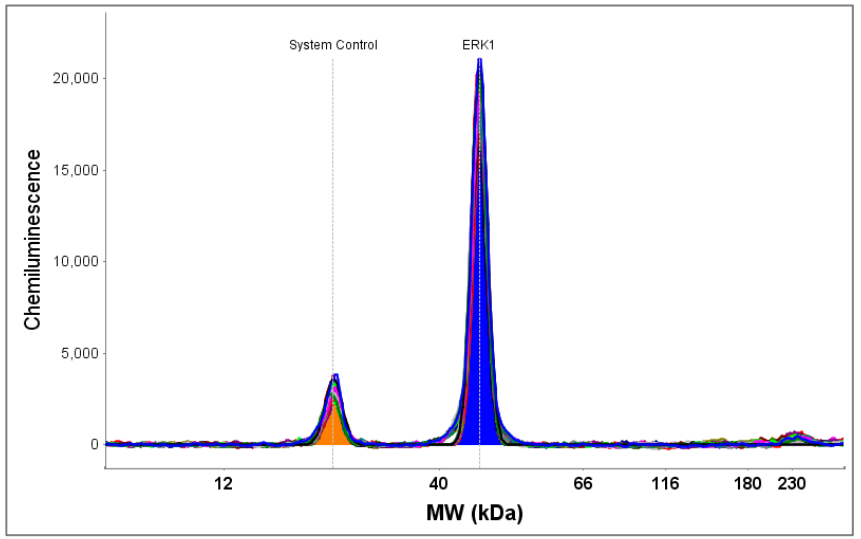
3. 运行
- 放毛细管卡盒
- 放加样板
- 运行程序

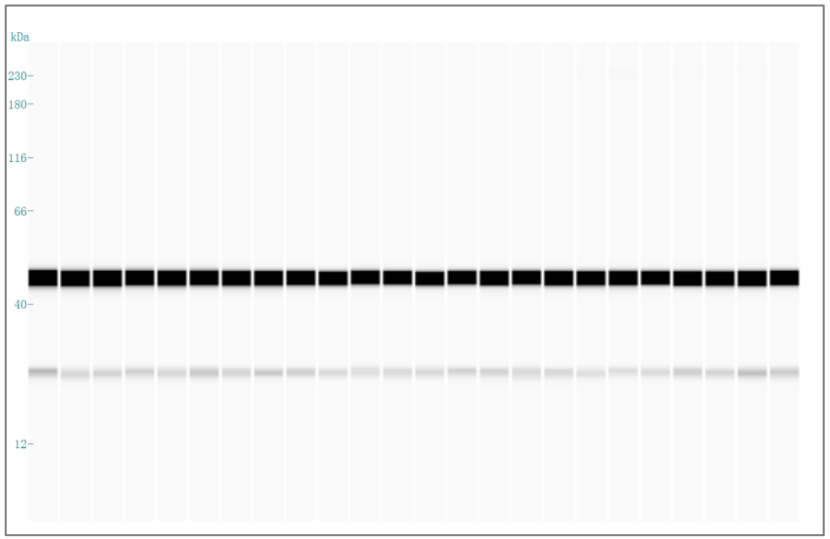
*样品不能加 Loading Buffer


THREE HOURS
LATER...

三种结果展现形式







成像图

毛细管实际曝光图

峰形图

峰面积为

化学发光信号值

泳道图

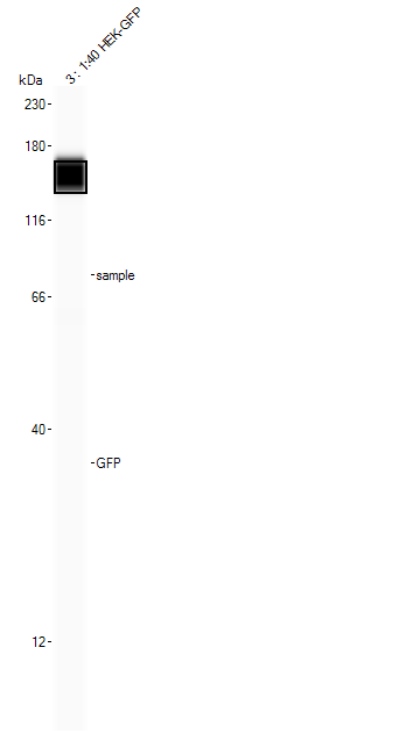
条带模拟图

第一次实现直接获得条带化学发光信号值

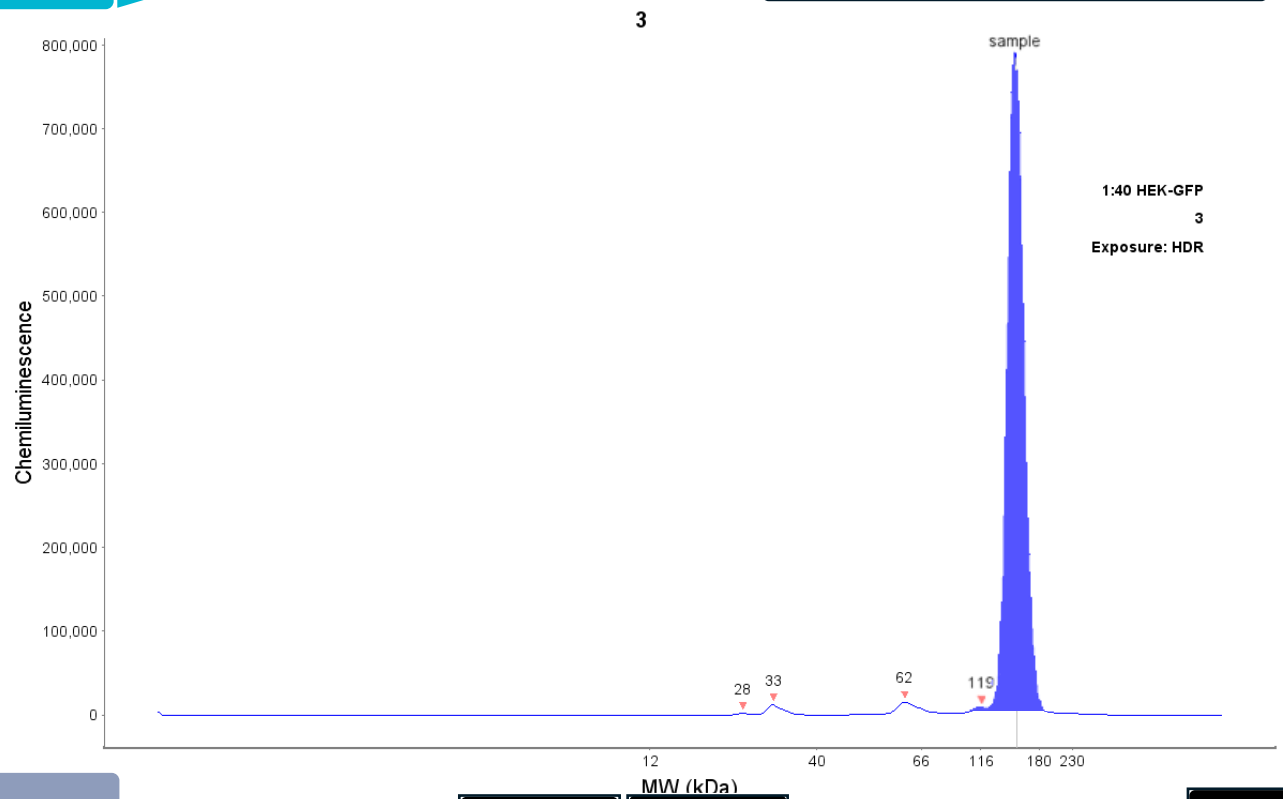
数字化WESTERN

1. Lane View

More Detail



2. Electropherogram View



3. More Rich Information

Sample	Primary	Cap	Peak	Name	Position	MW (kDa)	Peak Height: Height	Peak Area: Area	% Area	Conc (ng...	Width	Peak Area S/N: S/N	Baseline: Baseline
1:40 HEK-GFP	Gt anti...	3	1		422	28	2386.9	25194			9.9	84.5	308.4
1:40 HEK-GFP	Gt anti...	3	2		445	33	11712.6	170768			13.7	392.8	322.4
1:40 HEK-GFP	Gt anti...	3	3		540	62	16282.4	276943			16.0	422.1	386.7
1:40 HEK-GFP	Gt anti...	3	4		594	119	12403.2	178493			13.5	120.6	419.4
1:40 HEK-GFP	Gt anti...	3	5	sample	620	155	763025.7	9340434	100.0		11.5	29468.0	432.1

MEET SIMPLE WESTERN

JESS ABBY WES

GEL-RUNNING AUTO
TRANSFER-FREE
BLOT-FREE
HANDS-FREE

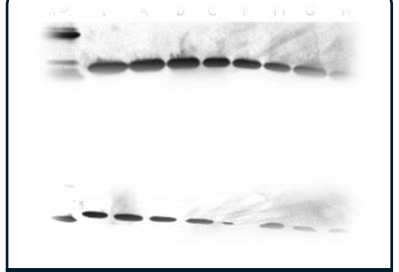
超微量样品+自动化+定量

1 Simple Western 工作原理

2 Simple Western 优势及应用

3 Simple Western 实验操作简介

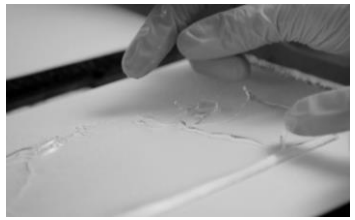
传统WB面临的挑战



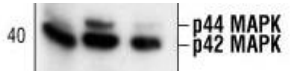
重复性差 1

- 5ug-20ug 总蛋白上样量

需要大量样本 2



大，小分子量蛋白 3

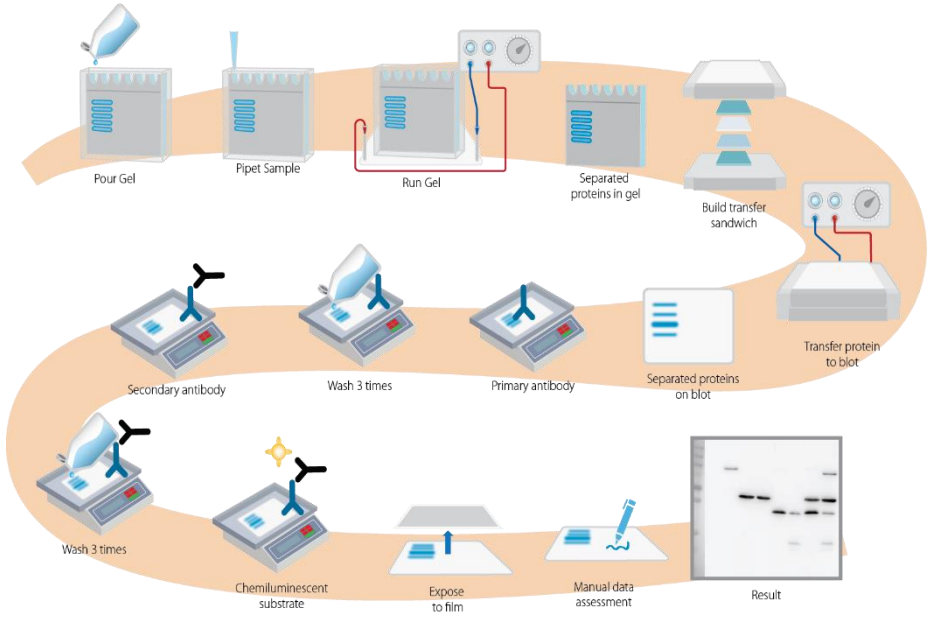


定量差 4

- 12-24 小时，手动操作多

周期长 5

1. SIMPLE WESTERN – 重现性好



The error in quantitative gel electrophoresis/Western blotting was investigated considering the purity testing of erythropoietin. The overall error was over 35% relative standard deviation. However, an analysis of variance elucidated that the interoperator variability was the dominant error source, which already explained almost 80% of the total variance. Careful compilation and investigation of the possible error sources strongly indicates that the immunoreaction after blotting and the subsequent color reaction are the major error sources in this case.

Roche **CV>35%**

Koller, A. and H. Watzig (2005). "Precision and variance components in quantitative gel electrophoresis." Electrophoresis **26**(12): 2470-2475.

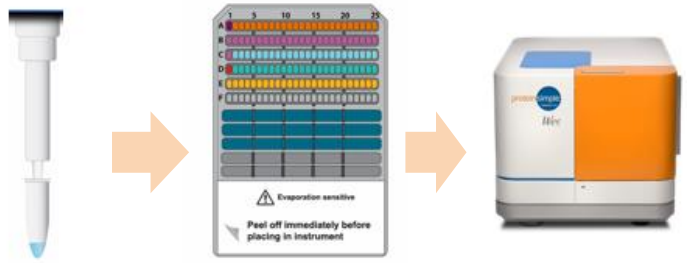


Table 1. Reproducibility (intermediate precision) of Simon™

Experiment	Concentration in µg/mL			
	Sample 1	Sample 2	Sample 3	
			p106	p86
Run 1	100	219	138	60
Run 2	102	256	138	53
Run 3	106	220	125	63
Run 4	111	242	131	53
Run 5	94	238	121	60
Mean	103	235	130	57
SD	7	16	8	5
%CV	6	7	6	9

MERCK **CV=9%**

Rustandi, R. R., et al(2012). "Qualitative and quantitative evaluation of Simon, a new CE-based automated Western blot system as applied to vaccine development." Electrophoresis **33**(17): 2790-2797.

2. SIMPLE WESTERN – 微量样品优势—分选干细胞

➢ **应用Wes完成2000个分选骨髓干细胞western blot**

The impact of integrin β2 on granulocyte/ macrophage progenitor proliferation

Li-Jie Zhang,[a] Cen Yan,[a] Sarah Schouteden,[b] Xiao-Juan Ma,[a] Dong Zhao,[a] Thorsten Peters,[c] Catherine M. Verfaillie,[b] Ying-Mei Feng [a,b]

Key Words. granulocyte/macrophage progenitors • integrin β2 • FcεRIα • GATA2 • proliferation

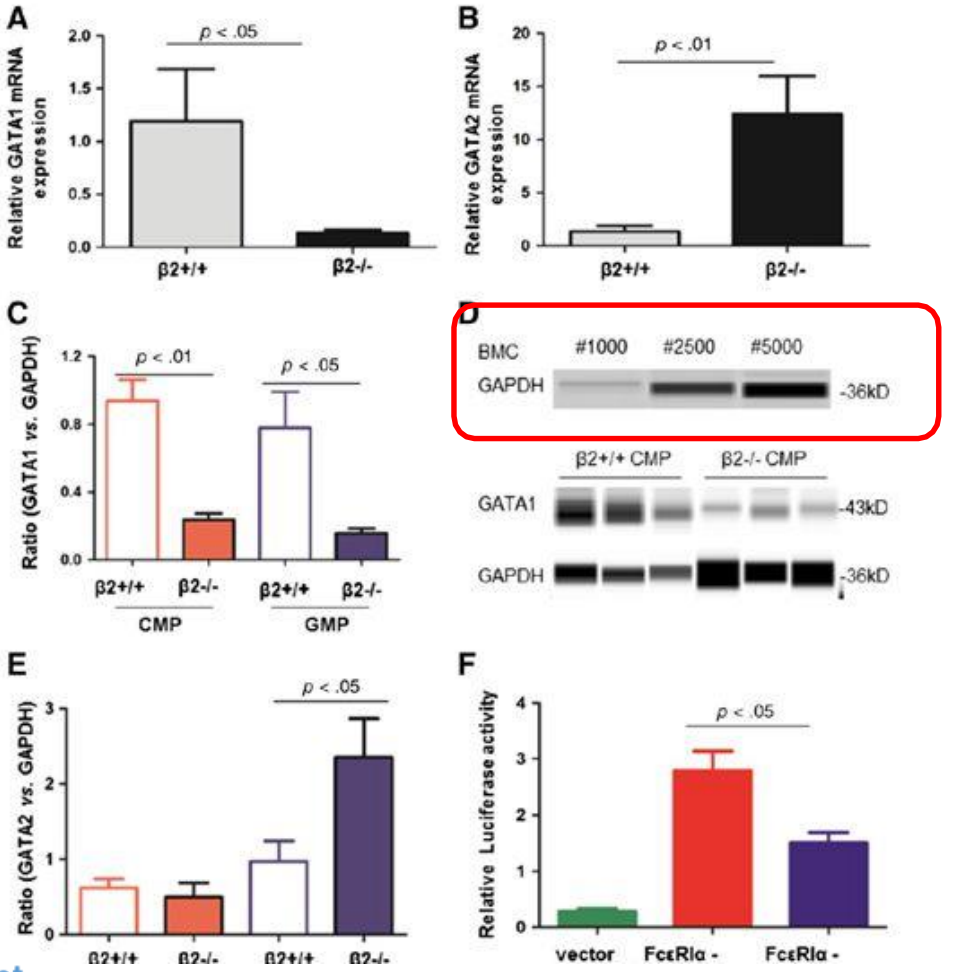
传统Western Blot检测：

一只小鼠流式分选获取骨髓干细胞量：1000个左右

1个孔需要数以万记细胞裂解的总蛋白，需要数十只小鼠，代价高昂。

Wes检测：1000个细胞（相等1只小鼠）即可检测到GAPDH信号，2500个细胞（相等于2-3小鼠）即可获得比较强且稳定的信号。

费用只有传统方法几十分之一



Transcriptional regulation of FcεRIα by GATA2. GMP were sorted out by **FACS**.
After protein concentration determination, equal amount of proteins was separated by capillary western blot.
GATA1, GATA2 and GAPDH expression were studied. **Each sample was pooled from 2 to 4 mice.**

The impact of integrin β2 on granulocyte/macrophage progenitor proliferation. **STEM CELLS 2018**

首都医科大学附属潞河医院内分泌中心
糖尿病防治北京市重点实验室

2. SIMPLE WESTERN – 微量样品优势—活检样本

nature

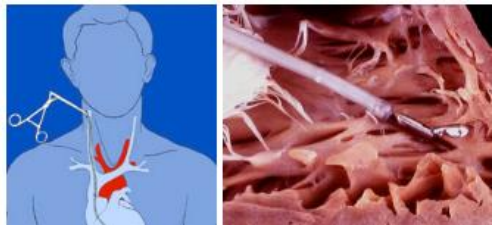
Article | Published: 10 April 2019

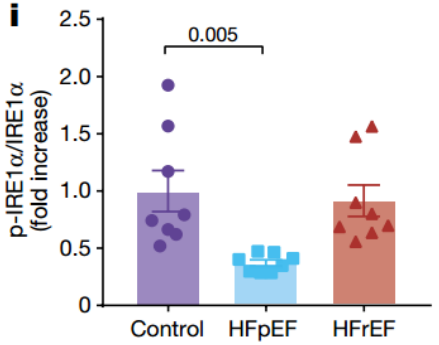
Nitrosative stress drives heart failure with preserved ejection fraction

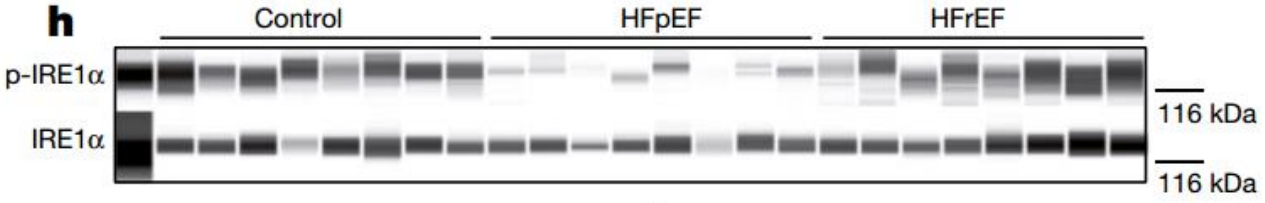
Gabriele G. Schiattarella[1,2], Francisco Altamirano[1], Dan Tong[1], Kristin M. French[1], Elisa Villalobos[1], Soo Young Kim[1], Xiang Luo[1], Nan Jiang[1], Herman I. May[1], Zhao V. Wang[1], Theodore M. Hill[1], Pradeep P. A. Mammen[1], Jian Huang[1], Dong I. Lee[3], Virginia S. Hahn[3], Kavita Sharma[3], David A. Kass[3], Sergio Lavandero[1,4,5], Thomas G. Gillette[1] & Joseph A. Hill[1,6]*

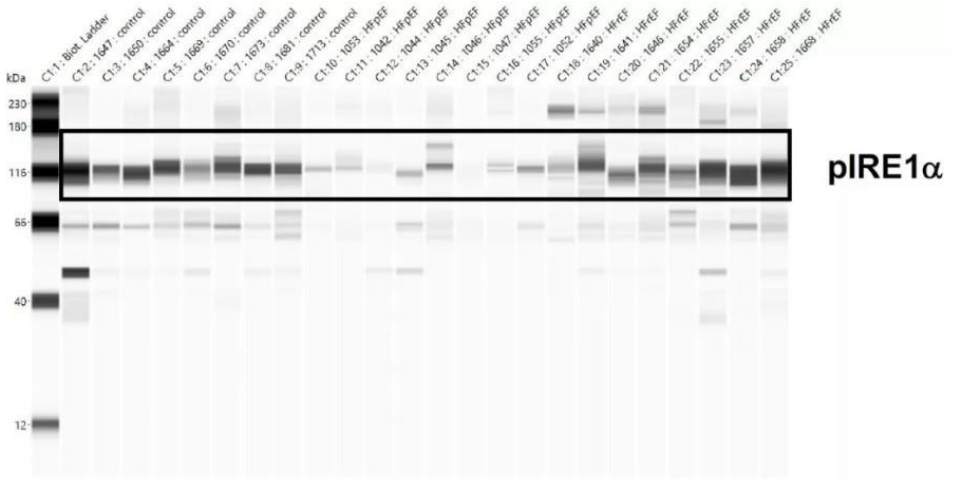
Wes检测人心脏活检样本IRE1α –XBP1信号通路蛋白

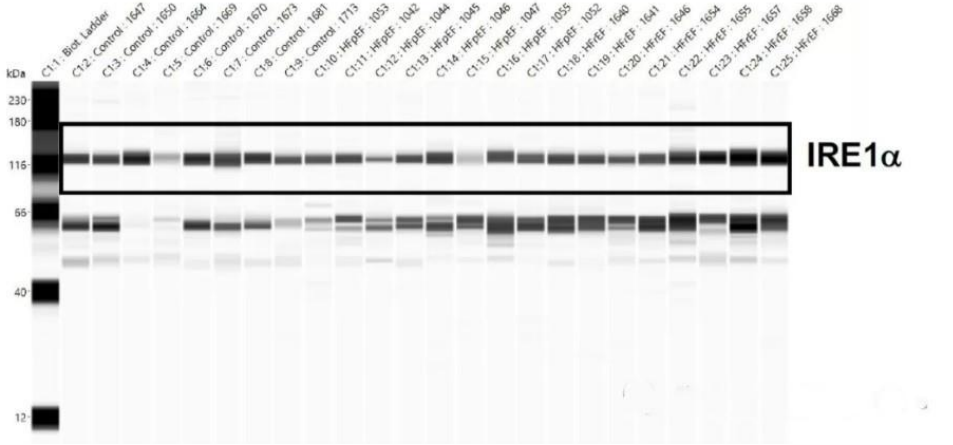
右心室心内膜活检术







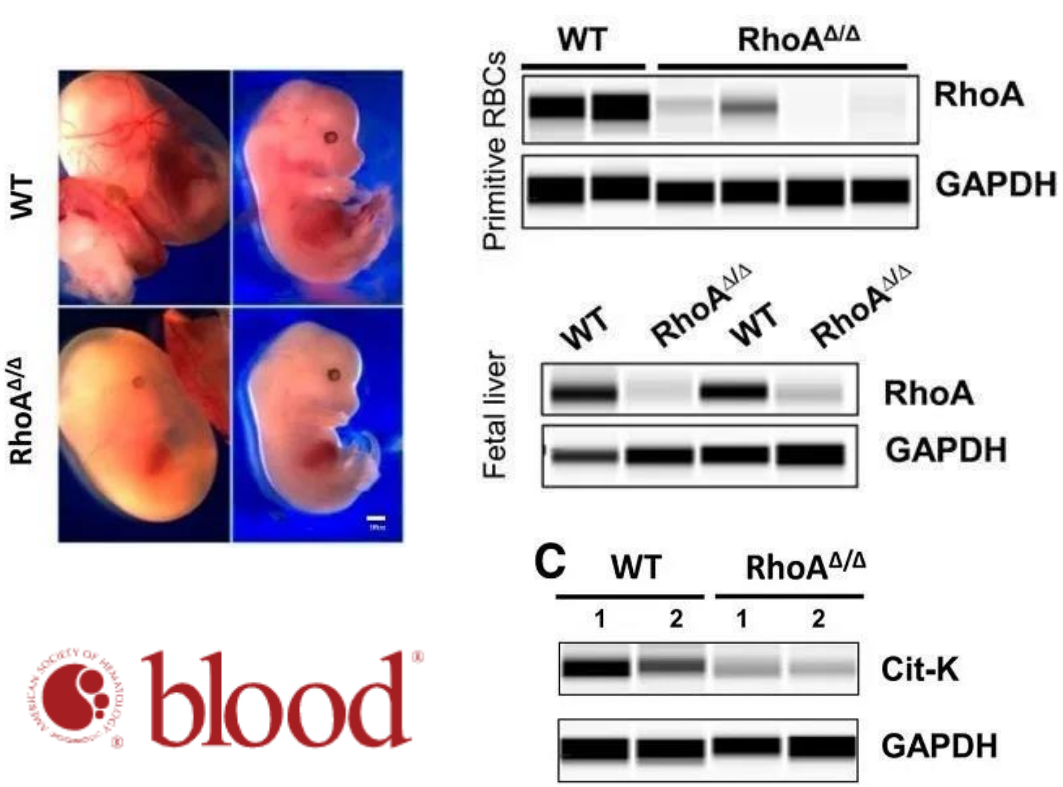




全膜western blot结果

2. SIMPLE WESTERN – 微量样品优势—胚胎来源样本

➢ 小鼠胚胎分选红细胞中分裂关键蛋白检测

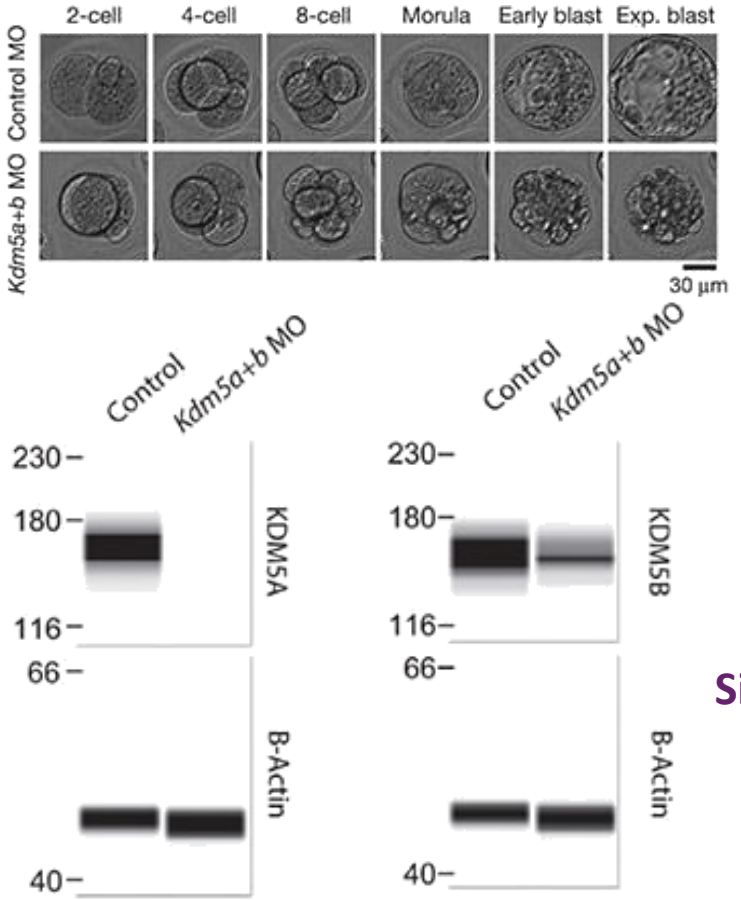


blood

美国辛辛那提大学辛辛那提儿童医学中心

Cytokinesis failure in RhoA-deficient mouse erythroblasts involves actomyosin and midbody dysregulation and triggers p53 activation. **BLOOD**

➢ 胚胎发育研究中组蛋白检测



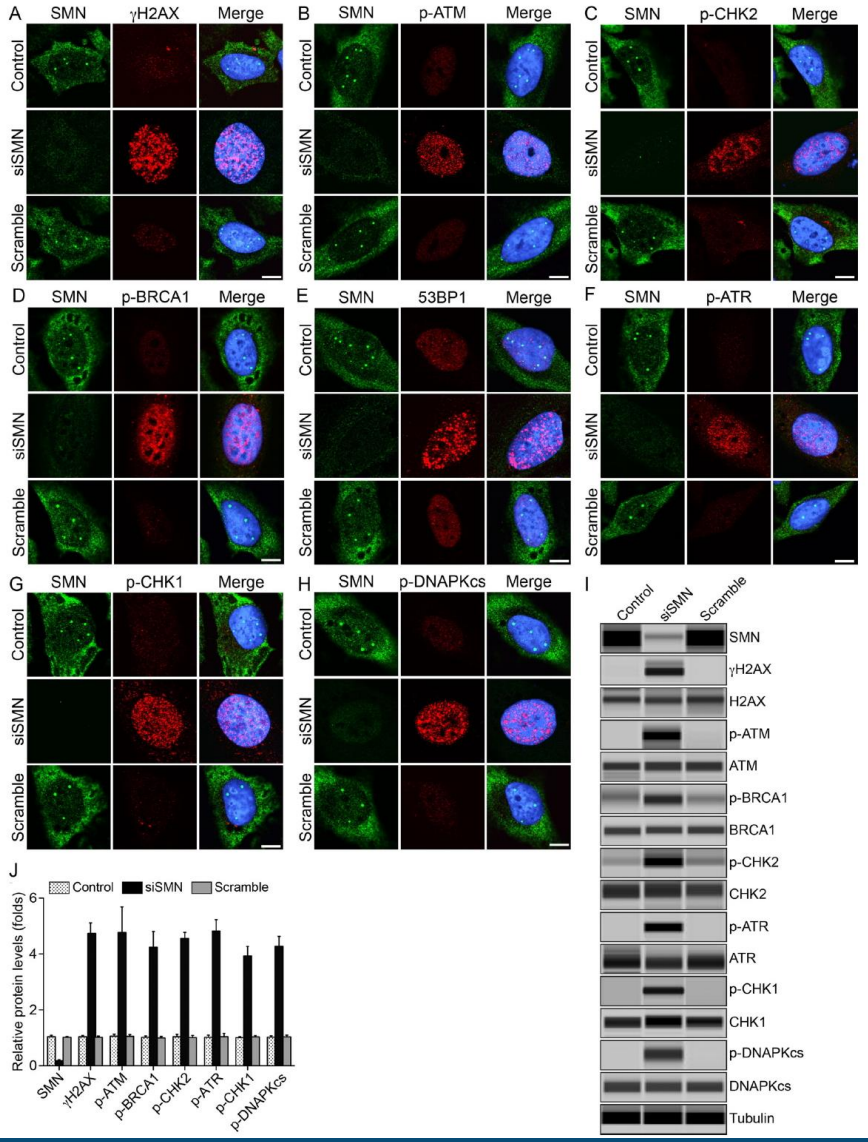
nature

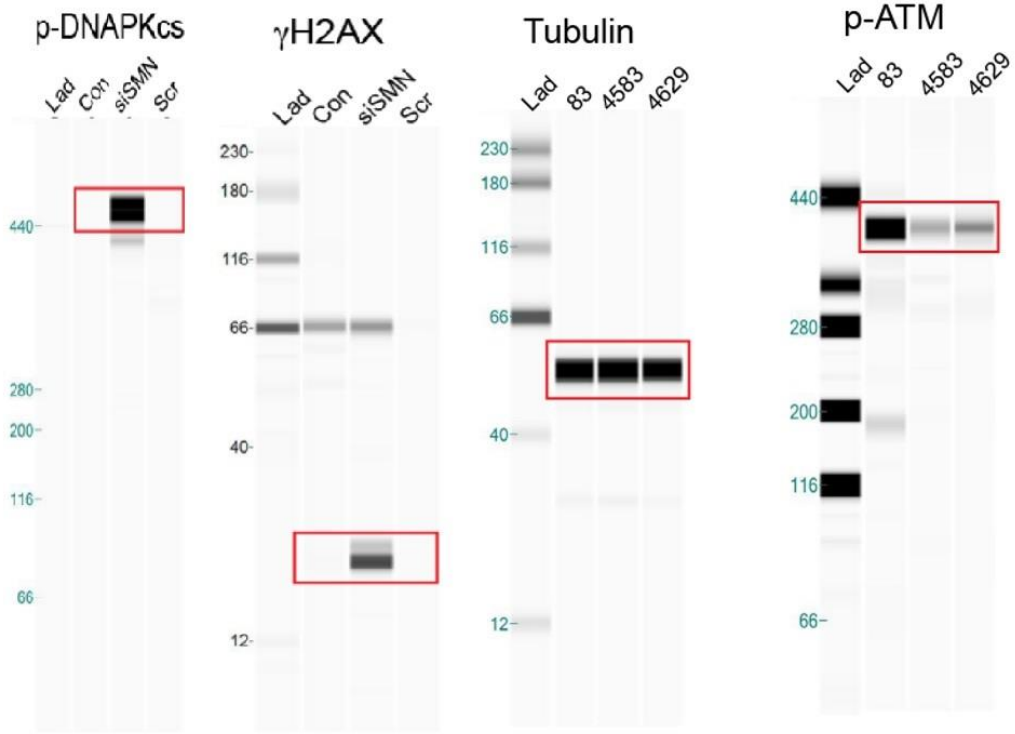
挪威奥斯陆大学附属医院
+
美国加州路德维格癌症研究所

132个2-cell胚胎中，得到7 ul的样品
Simple wes系统：上样3 ul，实际消耗40 nl。

Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic-transition, **Nature.(2016)**

3. SIMPLE WESTERN –蛋白质研究不再纠结分子量大小





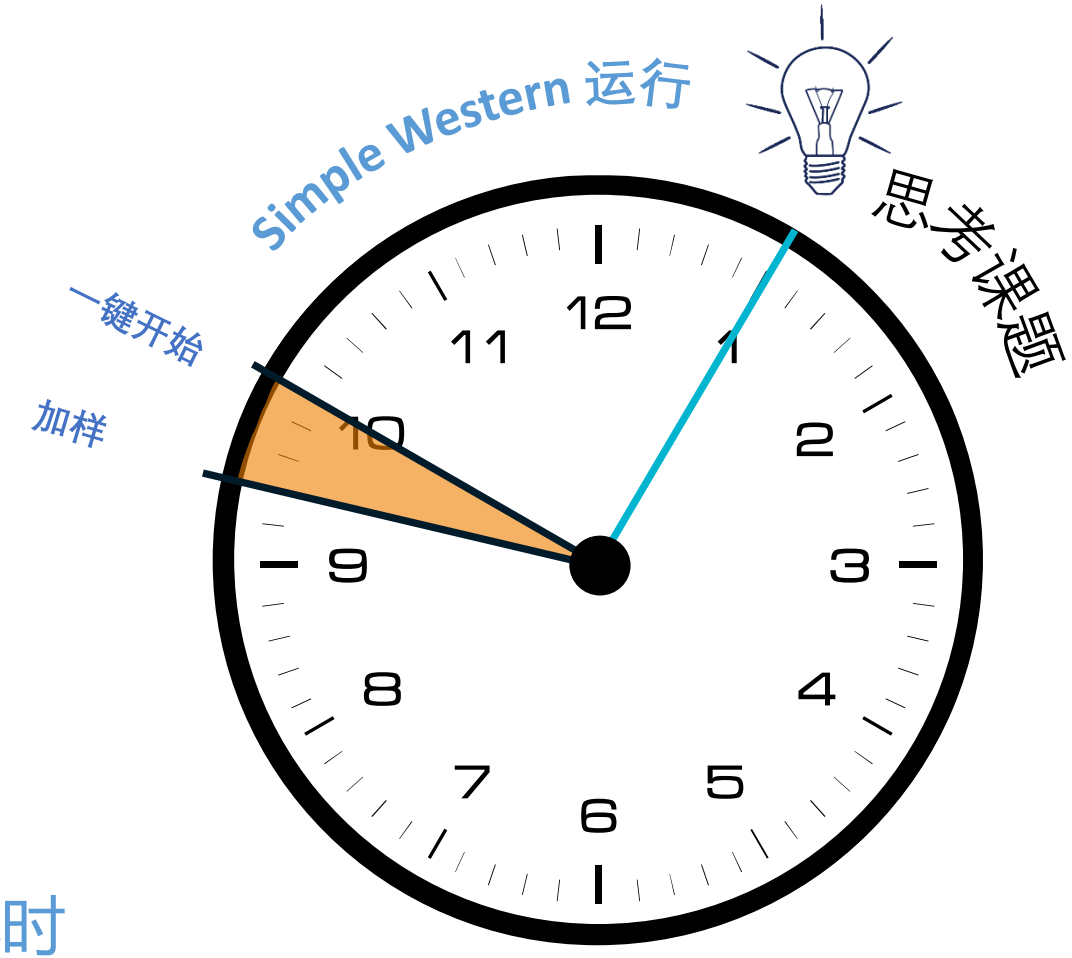
脊髓性肌萎缩（简称SMA疾病）相关蛋白：

SMN（40KD）DNAPKcs（450KD）CHK1（56KD）ATR（300KD）CHK2（62KD）BRCA1（220KD）ATM（350KD）H2Ax（15KD）rH2AX（15KD）SETX（35KD） Tubulin（55KD）

Combined deficiency of Senataxin and DNA-PKcs causes DNA damage accumulation and neurodegeneration in spinal muscular atrophy. Nucleic Acids Research, 2018

4. SIMPLE WESTERN – 3 H 完成实验

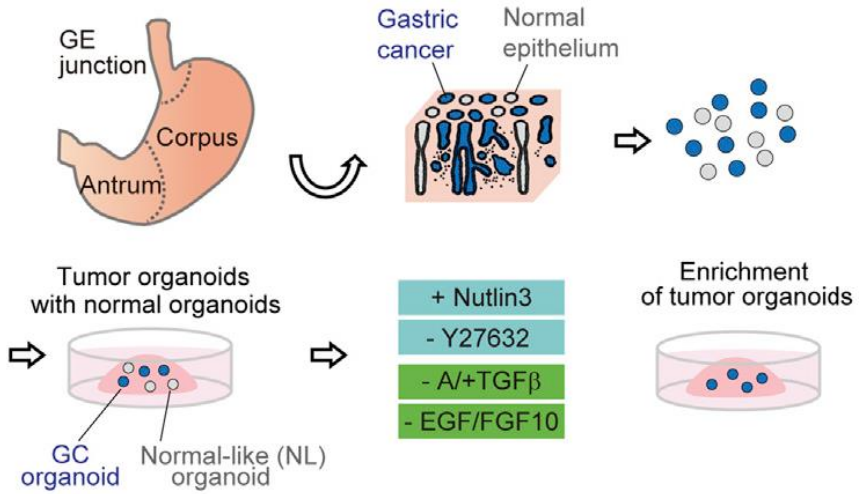
25 个样品
3个小时出结果

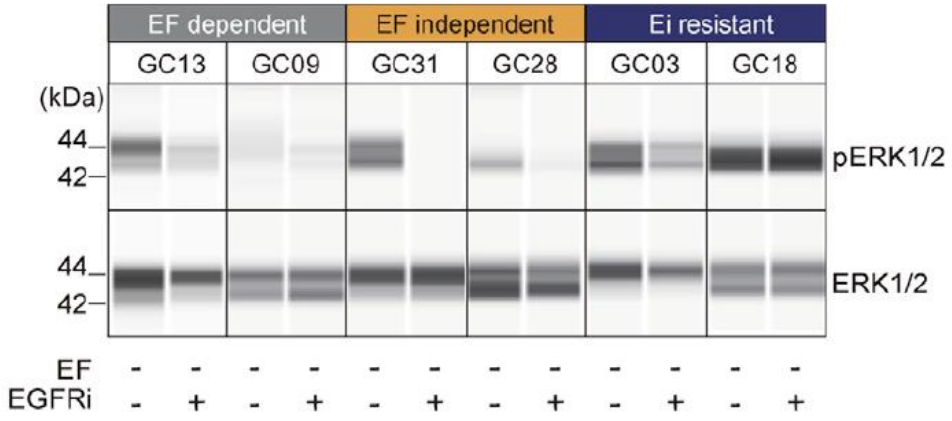


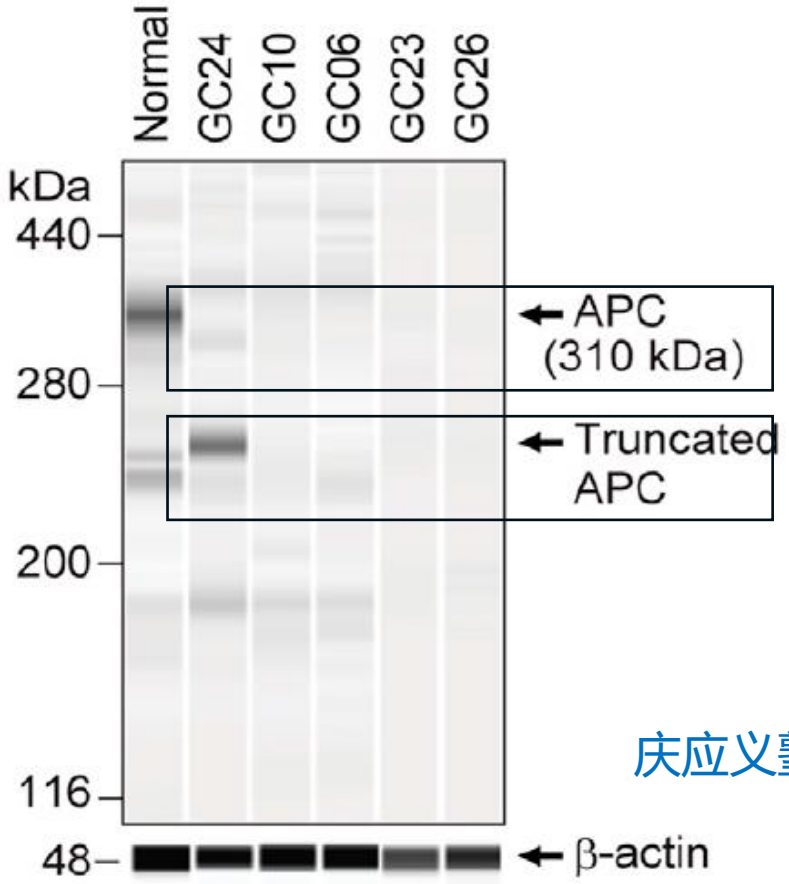
从开始运行到给出结果仅需 **3** 小时

人工操作时间仅 **0.5** 小时

5. SIMPLE WESTERN – 内参蛋白相对定量







胃癌类器官研究

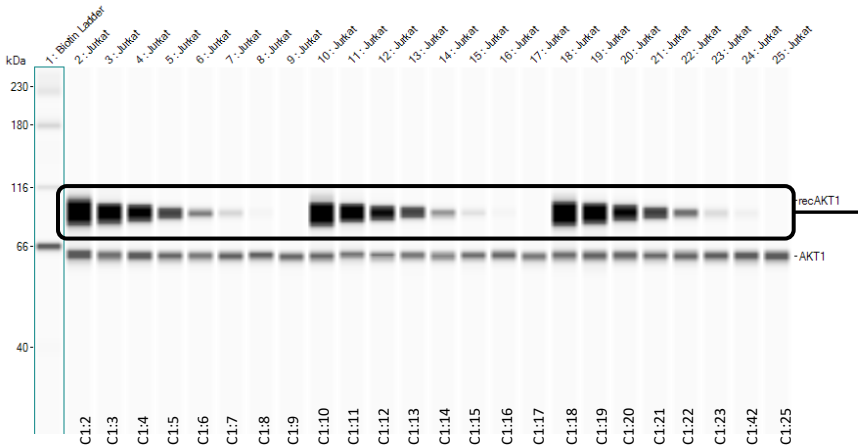
庆应义塾大学医学院消化内科

Divergent Routes toward Wnt and R-spondin Niche Independency during Human Gastric Carcinogenesis.
Nanki et al., *2018, Cell 174, 856–869* August 9, 2018

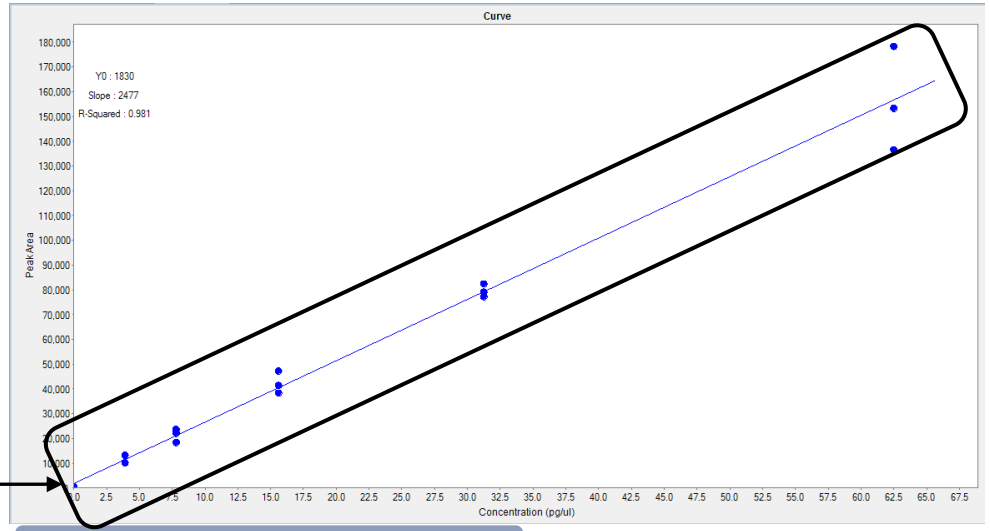
Cell

5. SIMPLE WESTERN – 绝对定量

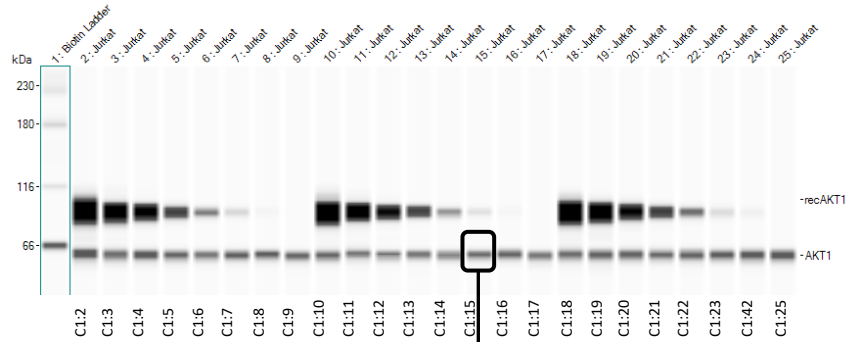
1. Confirm with Lane View



2. Create Standard Curve



3. Quantitate



Peaks | Capillaries

Sample	Prim...	Cap	Peak	Name	Positi...	MW (...	Height	Area	% Area	Conc ...	Width	S/N	Baseline
Jurkat	Anti...	C1:2	1	AKT1	496	64	4874.3	70846	14.4	27.86	13.7	23...	129.4
Jurkat	Anti...	C1:2	2	rec...	533	96	2980...	4209...	85.6	169.2	13.3	13...	142.6
Jurkat	Anti...	C1:3	1	AKT1	495	64	4193.2	58858	18.5	23.02	13.2	30...	132.9
Jurkat	Anti...	C1:3	2	rec...	533	95	2182...	2596...	81.5	104.1	11.2	16...	135.3
Jurkat	Anti...	C1:4	1	AKT1	495	64	4912.3	57228	24.3	22.37	10.9	23...	97.4
Jurkat	Anti...	C1:12	2	rec...	535	94	1320...	1365...	75.7	62.50	9.7	67...	123.3
Jurkat	Anti...	C1:13	1	AKT1	496	64	4235.3	44507	36.6	17.23	9.9	30...	113.4
Jurkat	Anti...	C1:13	2	rec...	534	95	7360.9	77229	63.4	31.25	9.9	52...	104.6
Jurkat	Anti...	C1:14	1	AKT1	498	63	3970.7	44205	53.6	17.11	10.5	30...	129.2
Jurkat	Anti...	C1:14	2	rec...	535	94	3681.9	38299	46.4	15.60	9.8	27...	129.9
Jurkat	Anti...	C1:15	1	AKT1	499	64	4238.4	45984	71.5	17.83	10.2	30...	107.4

GST标签的重组AKT蛋白

17.83 (pg/ul)

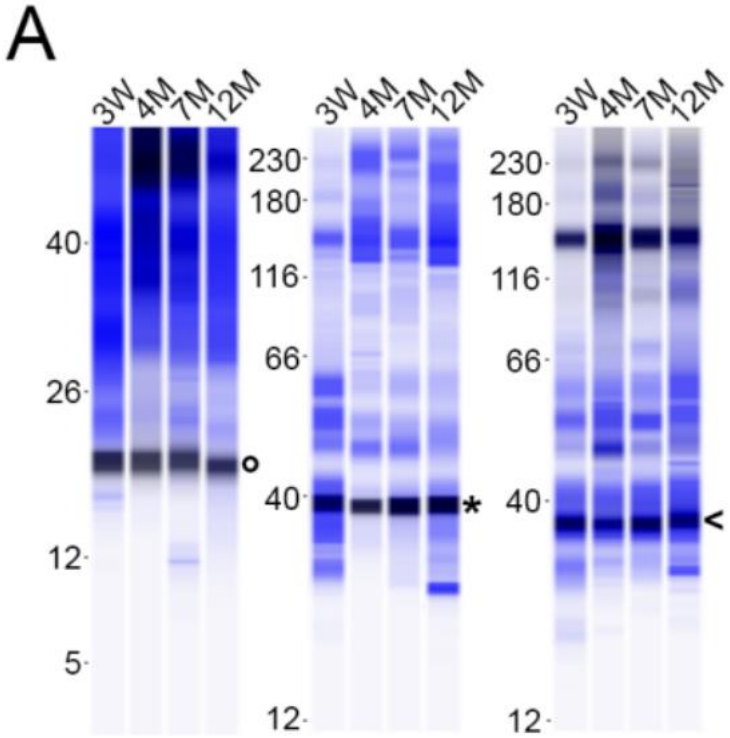
5. SIMPLE WESTERN – 总蛋白均一化

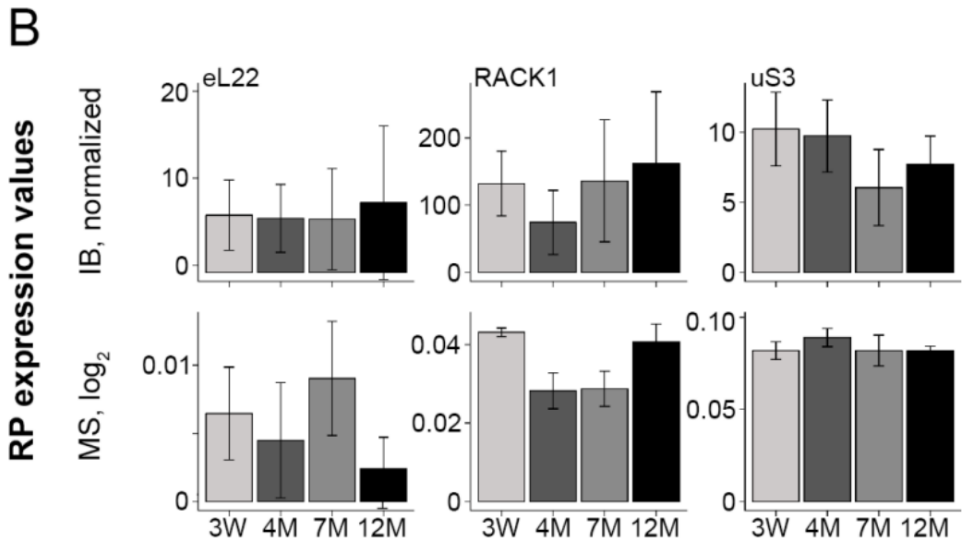
PNAS Proceedings of the National Academy of Sciences of the United States of America

➢ **PNAS：使用Jess总蛋白归一化技术定量研究核糖体蛋白**

德国汉堡大学
生物化学与分子生物学系

本研究利用Jess，对不同年龄的小鼠肝脏组织中的**核糖体蛋白（RPs）**进行定量，进一步从蛋白水平上，揭示了无论是**不同年龄的小鼠**，还是**小鼠不同组织**（脑组织亦或是肝脏组织），其核糖体蛋白的蛋白水平均保持不变。





Immunoblot Analysis. The immunoblot analysis was performed with liver samples using the capillary electrophoresis system (Jess, ProteinSimple). Frozen tissues (n = 4 to 6 biological replicates) were homogenized separately and fractionated in monosomal and polysomal fractions as prepared for the mass spectrometry analysis (see above) and loaded on the Jess separation module (2 to 40 kDa and 12 to 230 kDa). Antibodies against RACK1, uS3, and eL22 were purchased from Santa Cruz Biotechnology. The corresponding RP peaks were normalized to the total protein concentration using a protein normalization kit (ProteinSimple) and quantified using the Jess quantification module.

利用Jess的“总蛋白归一化”作为loading control，减少了不同样品间内参不平带来的相对定量误差。

Invariable stoichiometry ofribosomal proteins in mouse brain tissues with aging. ***Proc Natl Acad Sci*.2019** Nov 5;116(45):22567-22572.

6. 全膜 WESTERN BLOT

Data processing policy

Authors should make every attempt to reduce the amount of post-acquisitic processing may be unavoidable in certain instances and is permitted provic reflect that of the original. In the case of image processing, alterations mus brightness, contrast, color balance). In rare instances for which this is not p color channel on a microscopy image), any alterations must be clearly stat methods section. Groupings and consolidation of data (e.g., cropping of im and blots) must be made apparent and should be explicitly indicated in the comparisons should only be made from comparative experiments, and indi across multiple figures. In cases in which data are used multiple times (e.g performed simultaneously with a single control experiment), this must be cl and the methods section. In the event that it is deemed necessary for prop authors will be required to make the original unprocessed data available to manuscripts will be taken through a data presentation image screening pro

CellPress

Electrophoretic gels and blots

- Quantitative comparisons between samples on different gels/blots are strongly discouraged; if this is unavoidable, the figure legend must state that the samples derive from the same experiment or parallel experiments and that gels/blots were processed in parallel.
- Re-arranged lanes that are non-adjacent in the gel must be clearly indicated in a manner that delineates the boundary between the lanes. Re-arrangement of lanes should be stated as such in the figure legend.
- Loading controls (e.g. GAPDH, actin) must be run on the same blot. When sample processing controls are run on different gels, they must be identified as such in the figure legend. Cropped gels in the paper must retain all important bands.
- High-contrast gels and blots are discouraged, as overexposure may mask additional bands.
- Authors should take care to check their manuscripts for the following (1) check figures for duplications (2) check blots and gels for splicing of lanes (3) indicate whether panels are sample processing or loading controls (4) ensure that the unprocessed scans provided match the figures.

nature portfolio

6. 全膜 WESTERN BLOT

Contents lists available at ScienceDirect

Neurobiology of Aging

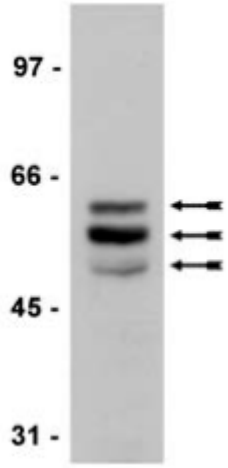
journal homepage: www.elsevier.com/locate/neuaging

稳定的出现的条带迁移，可能是新发现

Sustained high levels of neuroprotective, high molecular weight, phosphorylated tau in the longest-lived rodent

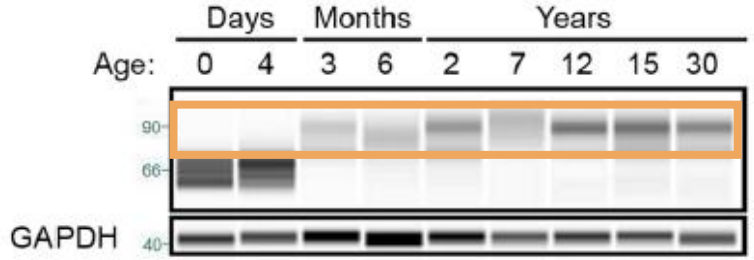
Miranda E. Orr, Valentina R. Garbarino, Angelica Salinas, Rochelle Buffenstein*

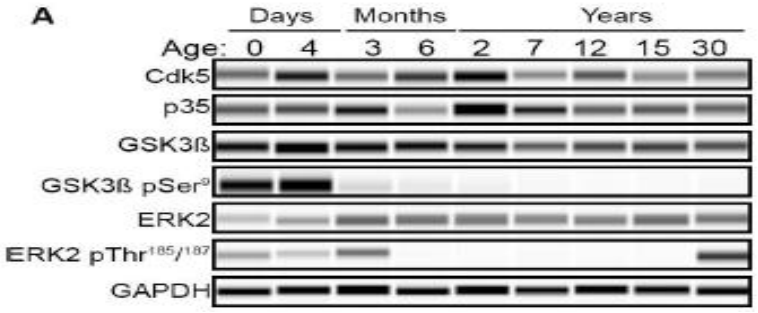
Department of Physiology and The Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

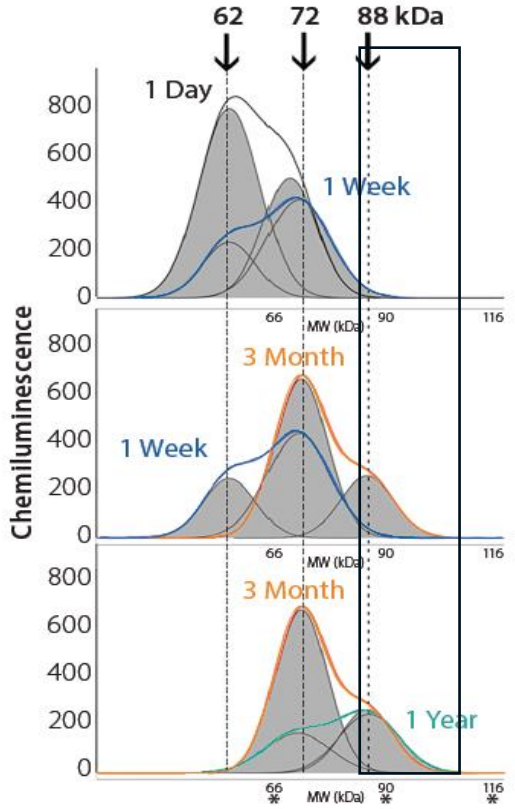


Specificity

Recognizes Tau (4-repeat isoform RD4), Mr 45-65 kDa. Higher MW band (68-72 kDa) represents phosphorylated Tau.

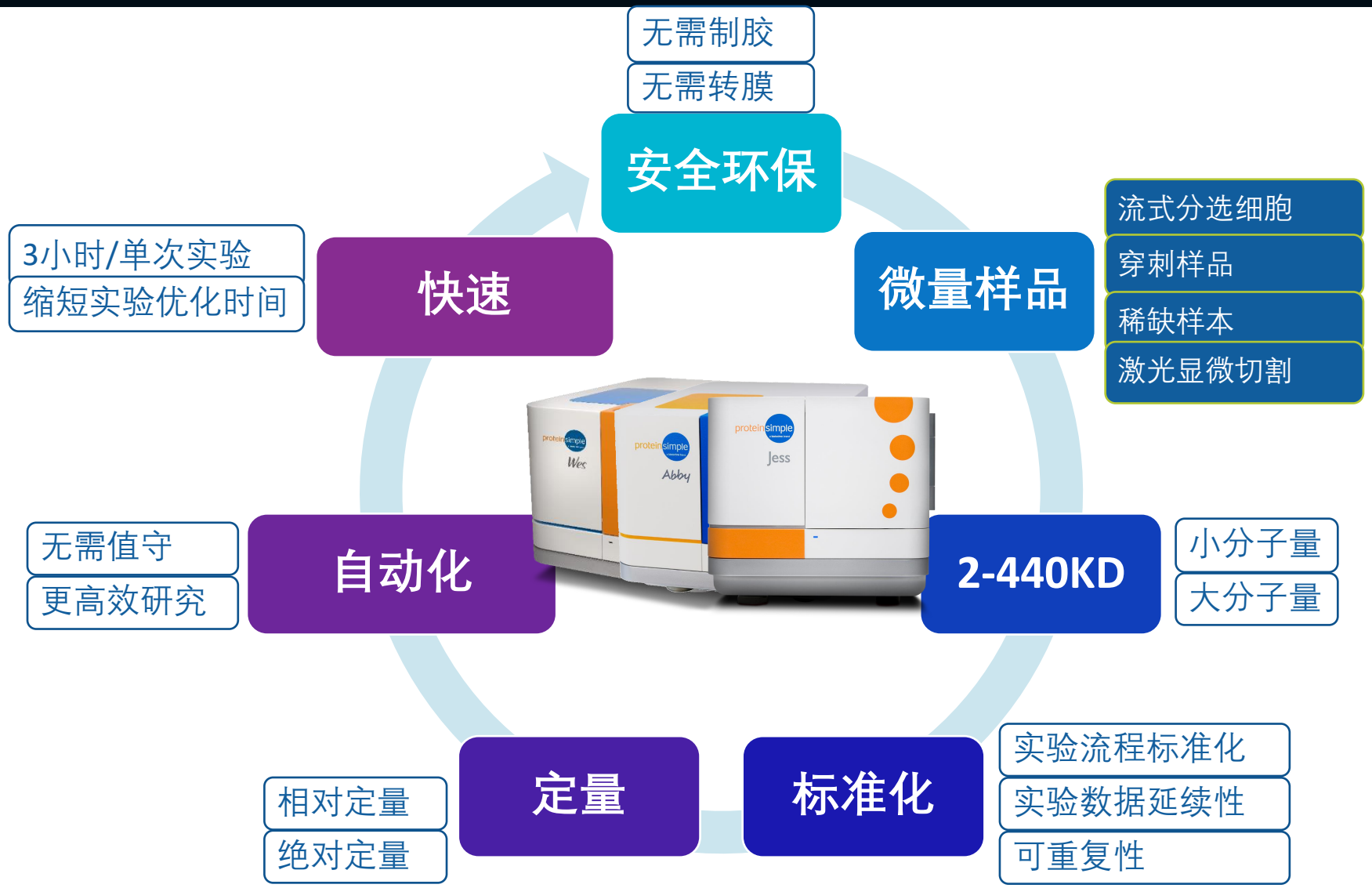






随着Rat 年龄增大，88KD蛋白出现，且在Tau蛋白中占的比例越来越高。

SIMPLE WESTERN 特点



MEET SIMPLE WESTERN

JESS ABBY WES

GEL-RUNNING AUTO
TRANSFER-FREE
BLOT-FREE
HANDS-FREE

超微量样品+自动化+定量

1 Simple Western 工作原理

2 Simple Western 优势及应用

3 Simple Western 实验操作简介

试剂盒

荧光检测试剂盒

Separation Module

- 2-40kDa分离试剂盒
- 12-230kDa分离试剂盒
- 66-440kDa 分离试剂盒
- 25 或 13 毛细管卡盒

Detection Module

- Anti-Rabbit NIR
- Anti-Mouse NIR
- Anti-Rabbit IR
- Anti-Mouse IR

RePlex Kit – RP-001

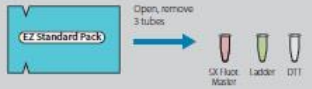
总蛋白均一化试剂盒

Protein Normalization Assay Module for Jess （AM-PN01)

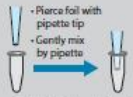
操作说明书

1. Assay Module preparation

A PREPARE STANDARD PACK REAGENTS

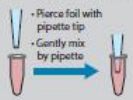


DTT (Clear Tube)



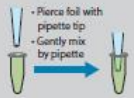
- Add 40 µL deionized water to make a 400 mM solution

Fluorescent 5X Master Mix (Pink Tube)



- Add 20 µL 10X Sample Buffer
- Add 20 µL prepared 400 mM DTT solution

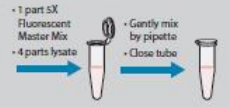
Biotinylated Ladder (Green Tube with Pink Pellet)



- Add 20 µL deionized water

B PREPARE YOUR SAMPLES

- The optimal protein concentration depends on the expression level of your protein. Dilute lysate as necessary with 0.1X Sample Buffer.
- Combine 1 part 5X Fluorescent Master Mix with 4 parts diluted lysate in a microcentrifuge tube (final concentration 0.4 mg/mL for chemiluminescence or 1.0 mg/mL for fluorescence). Produce enough diluted sample volume required for assay.



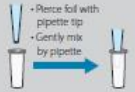
C DENATURE YOUR SAMPLES

D MIX LUMINOL-S AND PEROXIDE (IF APPLICABLE)

- Combine 200 µL Luminol-S and 200 µL Peroxide in a microcentrifuge tube

E PREPARE PROTEIN NORMALIZATION REAGENTS

- The Protein Normalization Reagent should only be prepared immediately before loading the plate.



- Prepare the Protein Normalization Reagent stock solution by adding 100 µL Protein Normalization Reconstitution Agent per tube. Thoroughly mix the reagent by pipetting 15 times.
- Use the following table to prepare the working solution of the reconstituted Protein Normalization Reagent stock solution. Thoroughly mix the working solution by pipetting 15 times.

PROTEIN MOLECULAR WEIGHT RANGE		
LYSATE CONCENTRATION	12–230 kDa	
	Stock Solution	Reconstitution Agent
0.2–1.2 mg/mL	50 µL	250 µL

2. Detection Module preparation

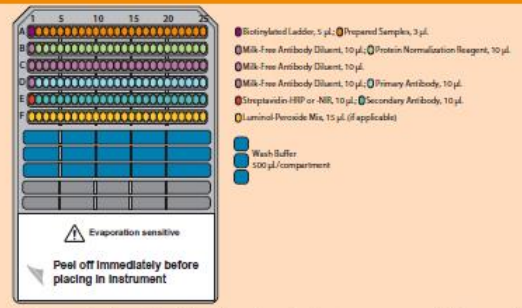
FOR CHEMILUMINESCENCE DETECTION

- Refer to Chemiluminescence Detection Module insert for instructions on primary and secondary antibody preparations

FOR FLUORESCENCE DETECTION

- Refer to Fluorescence Detection Module insert for instructions on primary and secondary antibody preparations

3. Pipette your plate

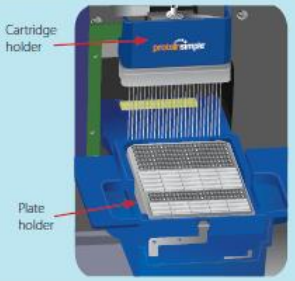


For more consistent results, keep the lid on the microplate between reagent additions and minimize bubble formation when adding Wash Buffer to the troughs and microplates. Protein Normalization reagent should be dispensed last.

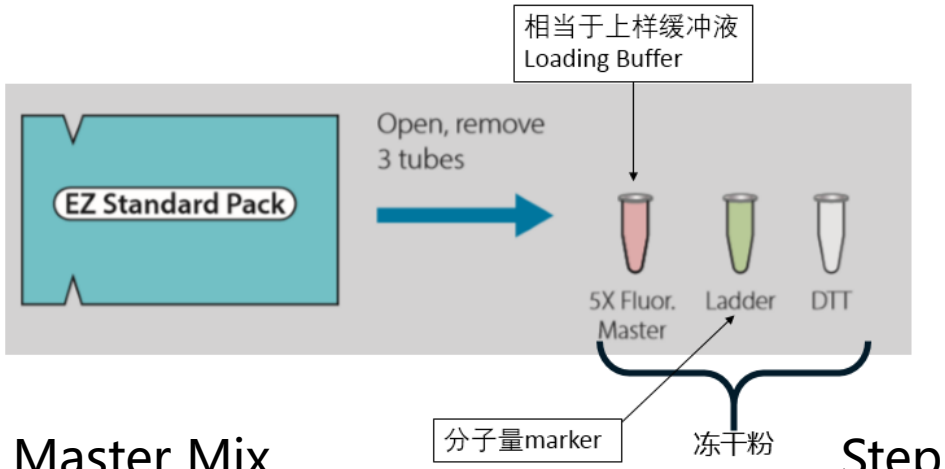
1. Dispense reagents into the assay plate using the volumes shown in the plate diagram.
2. Centrifuge the plate for 5 minutes at 2500 rpm (~1000 x *g*) at room temperature. Ensure liquid is fully down in all wells.

4. Start Jess

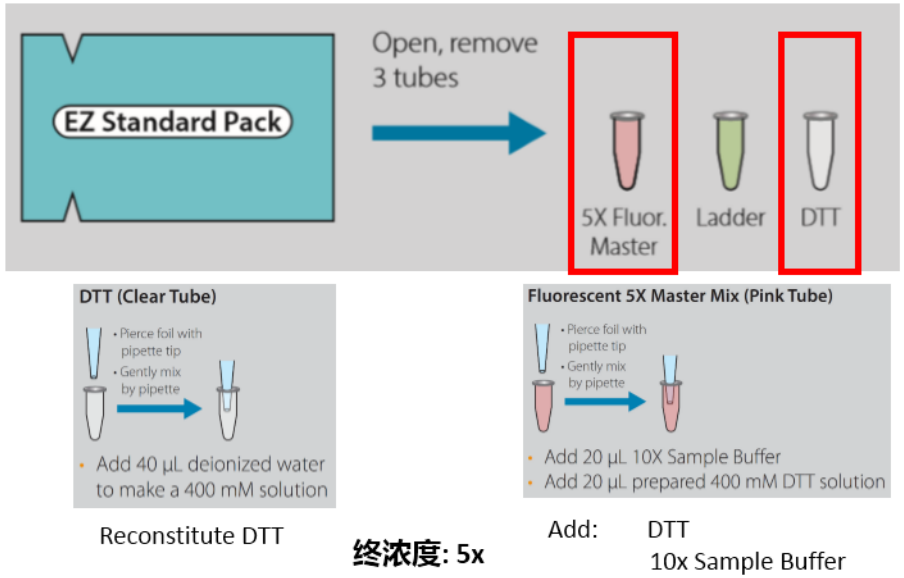
1. Select the desired assay parameters in Compass software.
2. Open Jess's door.
3. Insert a capillary cartridge into the cartridge holder. The interior light will change from orange to blue.
4. Remove the assay plate lid. Hold plate firmly on bench and carefully peel off evaporation seal. Pop any bubbles observed in the Separation Matrix wells with a pipette tip.
5. Place the assay plate on the plate holder.
6. Close Jess's door.
7. Click the Start button in Compass.
8. When the run is complete, discard the plate and cartridge.



样品制备



Step 1: 配制 5X FL Master Mix

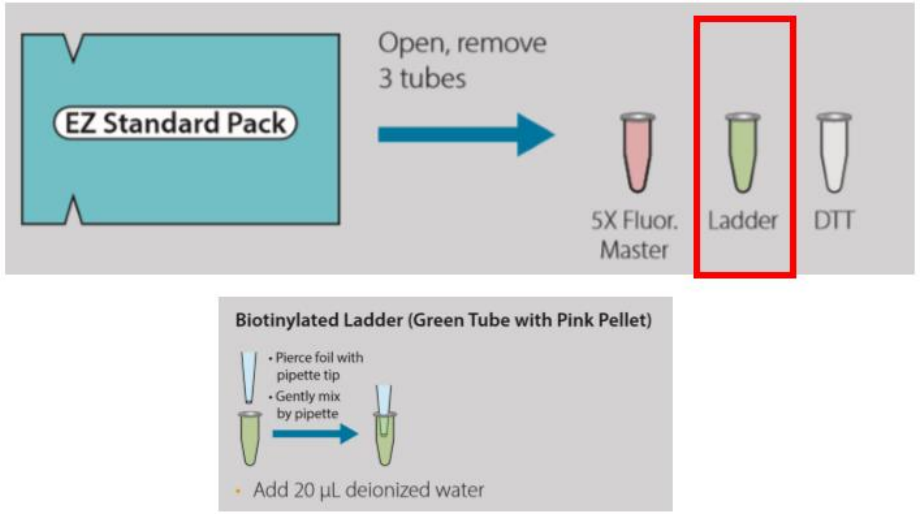


Reconstitute DTT

终浓度: 5x

Add: DTT
10x Sample Buffer

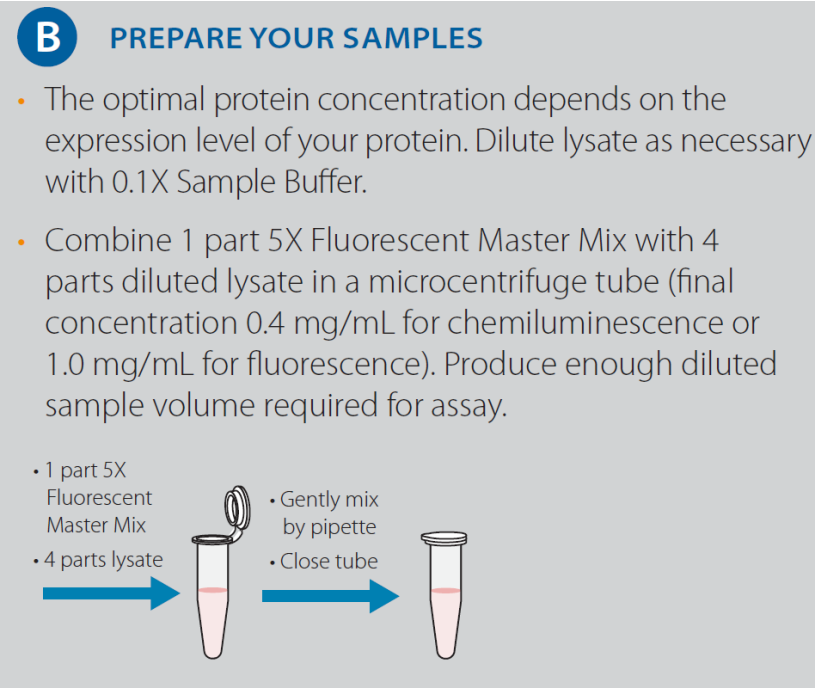
Step 2: 配制分子量标准品（marker）



Add: 20 µL H_2O

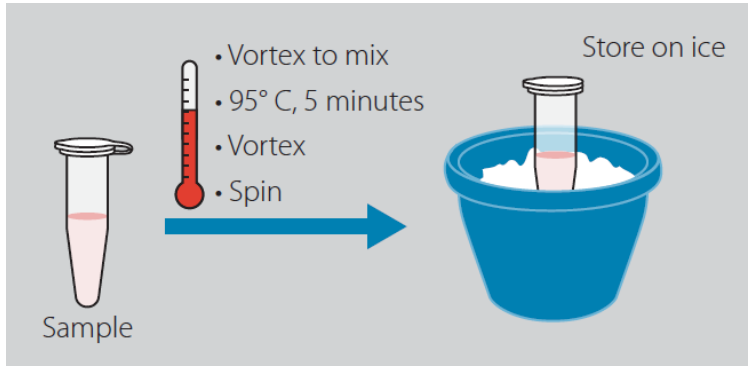
样品制备

Step 3: 稀释样品



样品用 **0.1X** Sample Buffer
（蛋白终浓度0.2-2mg/ml)

混合: **4** 体积 样品
1 体积 FL Master Mix



95°C 加热 5 分钟充分变性

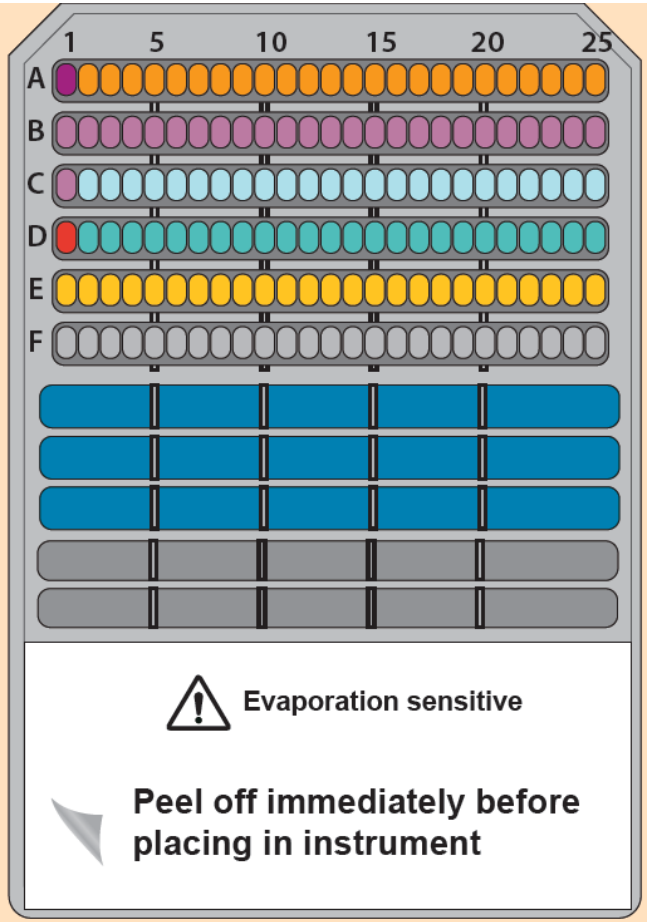
变性时建议用小管并封口以减少蒸发造成样品终浓度改变

- 需两次离心，以保证充分混合
  ① 变性后先冰上冷却
  ② Vortex混匀
  ③ 短时离心
  ④ Vortex混匀
  ⑤ 短时离心

检查 Proteinsimple 提供的 “Simple Western buffer compatibility table ”， 确认裂解液兼容性

板布局 — 化学发光

第 1 个孔:
分子量标准品 5 µL
封闭液 10 µL
封闭液 10 µL
Streptavidin HRP
发光底物 15 µL



Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL

Wes Antibody Diluent 2, 10 µL

Wes Antibody Diluent 2, 10 µL; Primary Antibody, 10 µL

Streptavidin-HRP, 10 µL; Secondary HRP Conjugate, 10 µL

Luminol-Peroxide Mix, 15 µL

Wash Buffer
500 µL/compartment
2.5 mL/row

分离胶
浓缩胶
洗胶液
电泳缓冲液
电泳缓冲液

样本:
A: Sample 3 µ
B: 封闭液 10 µL
C: 稀释后的一抗 10 µL
D: 二抗 10 µL
E: 发光底物 15 µL

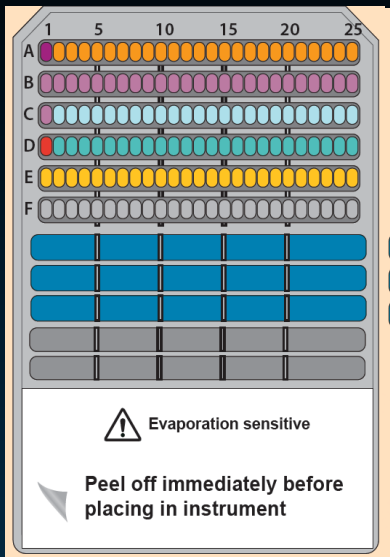
加样注意事项:

◆ 避免气泡：反向吸液

◆ 避免蒸发
- 操作连贯
- 加盖
- 上机才撕膜

◆离心: 室温，加盖

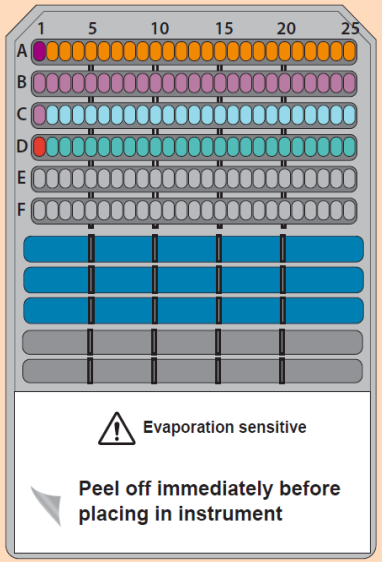
单独化学



- Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL
- Wes Antibody Diluent 2, 10 µL
- Wes Antibody Diluent 2, 10 µL; Primary Antibody, 10 µL
- Streptavidin-HRP, 10 µL; Secondary HRP Conjugate, 10 µL
- Luminol-Peroxide Mix, 15 µL

Wash Buffer
500 µL/compartment
2.5 mL/row

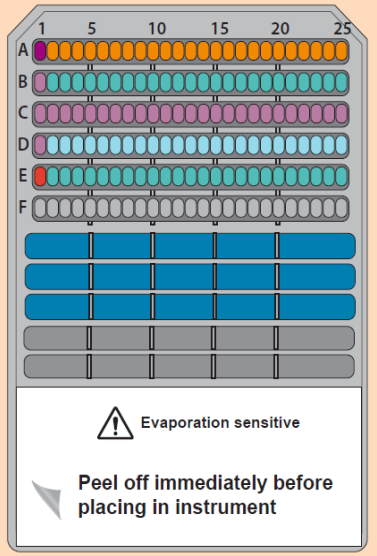
单独荧光



- Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL
- Milk-Free Antibody Diluent, 10 µL
- Milk-Free Antibody Diluent, 10 µL; Primary Antibody Multiplex Mix, 10 µL
- Streptavidin-NIR, 10 µL; Secondary Antibody Multiplex Mix, 10 µL

Wash Buffer
500 µL/compartment

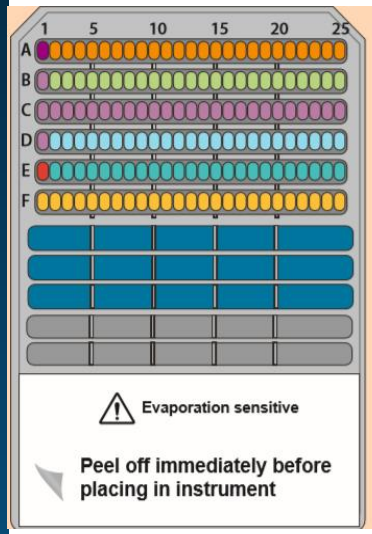
总蛋白和荧光



- Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL
- Milk-Free, 10 µL; Protein Normalization Reagent, 10 µL
- Milk-Free, 10 µL; Primary Antibody, 10 µL
- Streptavidin-NIR, 10 µL; Secondary NIR/IR Conjugate, 10 µL

Wash Buffer
500 µL/compartment

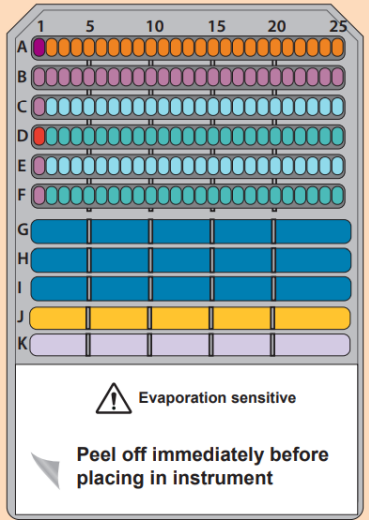
三者结合



- Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL
- Milk-Free Antibody Diluent, 10 µL; Protein Normalization Reagent, 10 µL
- Milk-Free Antibody Diluent, 10 µL
- Milk-Free Antibody Diluent, 10 µL; Primary Antibody, 10 µL
- Streptavidin-HRP or -NIR, 10 µL; Secondary Antibody, 10 µL
- Luminol-Peroxide Mix, 15 µL (if applicable)

Wash Buffer
500 µL/compartment

两轮免疫

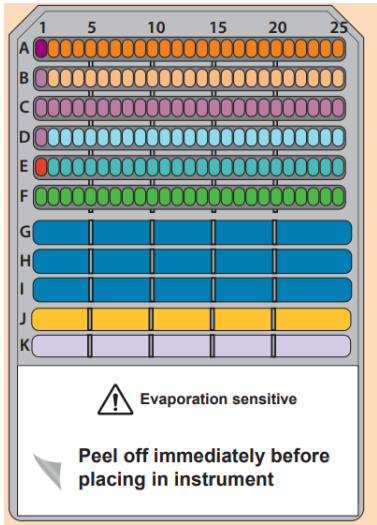


- Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL
- Antibody Diluent (2 or Milk-Free), 10 µL
- Antibody Diluent (2 or Milk-Free), 10 µL; Primary Antibody for Probe 1, 10 µL
- Streptavidin-HRP or NIR, 10 µL; Secondary Antibody for Probe 1, 10 µL
- Antibody Diluent (2 or Milk-Free), 10 µL; Primary Antibody for Probe 2, 10 µL
- Antibody Diluent (2 or Milk-Free), 10 µL; Secondary Antibody for Probe 2, 10 µL

Wash Buffer
500 µL/compartment

- Luminol-Peroxide Mix, 170 µL/compartment (for chemiluminescence only)
- RePlex™ reagent mix, 300 µL/compartment

免疫和总蛋白



- Biotinylated Ladder, 5 µL; Prepared Samples, 3 µL
- Antibody Diluent (2 or Milk-Free), 10 µL; Total Protein labeling reagent, 10 µL
- Antibody Diluent (2 or Milk-Free), 10 µL
- Antibody Diluent (2 or Milk-Free), 10 µL; Primary Antibody for Probe 1, 10 µL
- Streptavidin-HRP or NIR, 10 µL; Secondary Antibody for Probe 1, 10 µL
- Total Protein Streptavidin-HRP for Probe 2, 8 µL

Wash Buffer
500 µL/compartment

- Luminol-Peroxide Mix, 170 µL/compartment
- RePlex™ reagent mix, 300 µL/compartment

公众号报修流程

普诺森生物科技

PROTEINSIMPLE
SIMPLE YOUR PROTEIN ANALYSIS

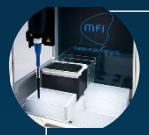
4000-863-973

FluorChem
- **Simple** Imaging

Jess/Wes/Abby
- **Simple** Western

Ella
- **Simple** ELISA

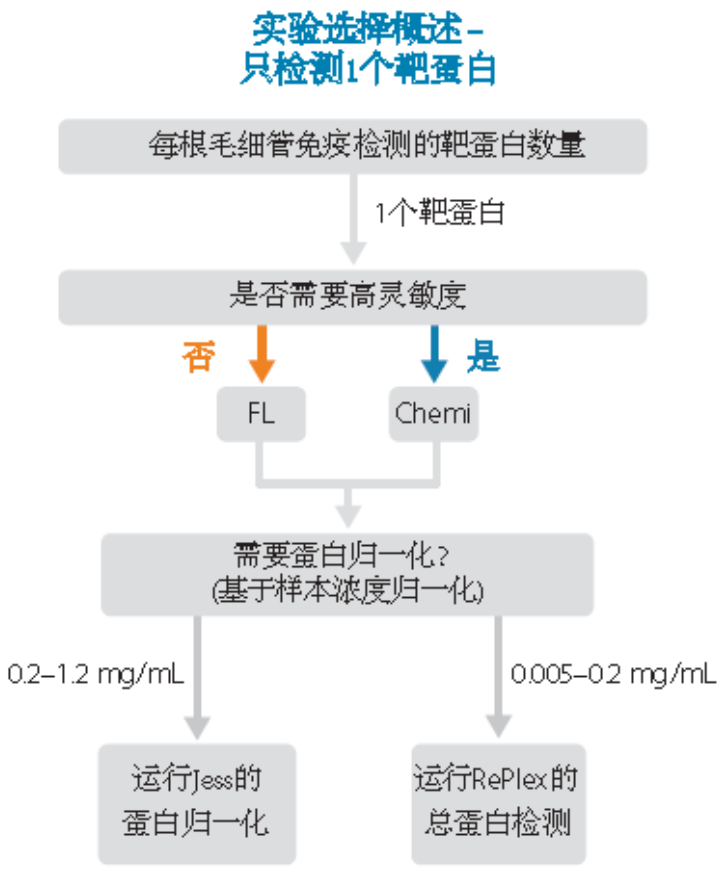
Milo
- **Simple** Sc-Western

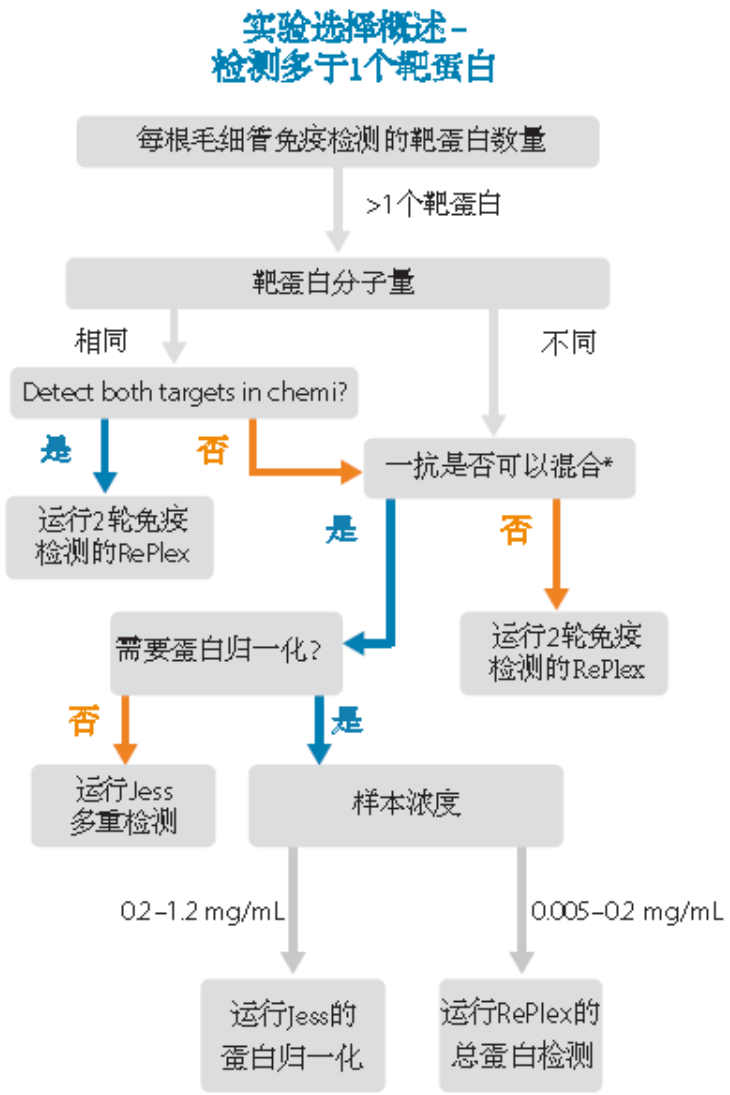


MFI
- **Simple** Particle Analysis

Maurice
- **Simple** icIEF + CE-SDS

JESS实验决策树





RePlex：如果第二轮进行化学发光检测，推荐第一轮进行低丰度靶蛋白的化学发光或荧光检测。
多重检测：多种一抗混在一起进行孵育后检测，需验证可行性。
*检查将要混合在一起的抗体各自的交叉反应、动态范围以及背景。